RADIATION-INDUCED NUTRITIONAL AND MORPHOLOGICAL MUTANTS OF GLOMERELLA[1]

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MUTATIONS have been readily induced in various organisms by ionizing radiations, by ultraviolet light, and most recently by several chemical compounds. The diverse nature of these mutagenic agents suggests that they might produce different kinds of mutations or, perhaps, different proportions of the same kinds of mutants. The discovery of any such differences in mutagenic action might provide valuable clues concerning the nature of the mutation process itself. The increasing use of mutagens to induce nutritional deficiencies in microorganisms has produced a considerable quantity of data on the comparative genetic effects of the principal mutagenic agents, but as yet no striking qualitative differences in mutagenic effect have emerged from these data (TATUM 1950).

However, the data do make possible preliminary comparisons between different organisms with respect to the relative frequencies of the various kinds of mutants which have been found in them. Thus the relative stabilities of those genes which control apparently homologous syntheses in different organisms may be studied along with the variations in the genetically controlled metabolic pathways which are used by different organisms to synthesize the same metabolite. In addition to the genetic and biochemical significance of such data, the data should also be useful in helping to elucidate evolutionary relationships.

The present report presents the results of an extensive investigation of the comparative mutagenic effects of X-rays, fast neutrons, and ultraviolet radiation on the fungus, Glomerella. Comparisons are also made between the mutations which have been found in Glomerella, Neurospora, and Salmonella.

MATERIALS AND METHODS

Characteristics of Glomerella

The induction of mutations in Glomerella and their investigation from a genetic and biochemical point of view have previously been reported (MARKERT 1950). Further references to the genetics of Glomerella may be obtained from WHEELER (1950). Glomerella possesses characteristics which make it especially suitable for genetic investigations. It is an ascomycete with simple, defined nutritional requirements; the wild type will grow either in a liquid medium or on a solid agar medium containing only inorganic salts and an

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organic source of energy such as sucrose. The Glomerella stock (referred to as standard type) most frequently used in this investigation differs from the wild type by gene changes at two loci and is symbolized by A^1B^1 (*cf.* WHEELER 1952, for terminology). The wild type (A^+B^+) is usually the type isolated from nature and produces perithecia in glomerate masses. The A^1B^1 stock produces no perithecia but on an appropriate medium (WESTERGAARD 1947) produces abundant conidia dispersed more or less evenly over the mycelium. These conidia are all haploid and uninucleate. The conidia are about 16 μ in length and are apparently covered by a soluble, slightly sticky substance that prevents them from being readily airborne, and therefore, laboratory contamination by Glomerella is negligible. After germination on a minimal or complete agar medium (*cf.* MARKERT 1950, for media) these uninucleate conidia produce small colonies which in Petri dish cultures may be easily counted and then transferred to culture tube agar slants. The growth rate of Glomerella is convenient for laboratory manipulation. About 30 to 36 hours at 25°C suffice for germination of conidia and formation of colonies which are large enough to be counted readily or transferred to agar slants. An additional four or five days of growth on agar slants produce a large enough colony with sufficient differentiation to permit a final evaluation of its visible hereditary characteristics.

A genetic analysis of mutant colonies may be made after crossing complementary sexual types (MARKERT 1949; WHEELER 1952) by plating out ascospores from crushed perithecia and picking colonies at random or by isolating single spores from individual asci as in Neurospora. However, since the average size of ascospores is about 23 × 5 microns, the isolation of individual spores is a difficult task without the aid of a micromanipulator. Crossing and the formation of mature ascospores require one to two weeks. In addition, the genetic analysis of an individual mutant requires at least eight days.

The fungus mycelium, which grows as a dense felt-like mat on the surface of an agar culture medium, is a sensitive reflector of genetic change. Genetic changes are indicated by variations in pigmentation, morphology, or growth rate. Pigmentation may vary from white through various shades of gray to black with red, orange, yellow, brown, and even greenish hues added in genetically determined amounts. A multitude of distinct morphological variants may be recognized, and variations in growth rate of about 10 percent or more can be satisfactorily determined. The mutability is such that at appropriate doses of irradiation more than 50 percent of the surviving colonies may be recognized as mutant.

Selection of mutants

The kind of mutants recovered and identified from irradiated conidia depends upon the selection procedure used. In these investigations the irradiated spores were plated out in an agar medium containing inorganic salts, sucrose, and extracts of malt and yeast (referred to as complete medium). After germination and colony formation, small mycelial transplants were made to the center of agar slants (6 ml in 15 × 180 mm culture tubes) containing

the same complete medium. It is necessary to transfer all of the colonies which develop on any plate since the late-germinating spores produce a higher percentage of mutants than do the early-germinating spores in contrast to the results obtained with Neurospora (SANSOME *et al.* 1945). In practice, colonies were picked from each plate on both the second and third days following the plating out of irradiated spores.

At four days and at six days of growth on the agar slants at 25°C an evaluation of all the colonies was made. Those which were visibly different from the control cultures at either time were classified as mutant and then assorted into four groups: 1) moribund, 2) morphological, 3) pigment, and 4) retarded growth mutants. The moribund colonies were those which grew to a diameter of 1 or 2 mm on complete medium and then died. Apparently a genetic deficiency, irreparable on complete medium, had been induced in these mutants. The limited growth achieved by these moribund mutants was perhaps due to reserve material contained in the spore at the time of irradiation. Only the viable mutants will be discussed further in this report. The retarded growth mutants achieved about 80 percent or less of the growth of control colonies in the same period of time.

Since mutants frequently differed from the standard cultures in more than one of the four evaluative characteristics, it was necessary to employ a system of priority in order to establish mutually exclusive groups. The order of priority used is that listed above; thus a morphological mutant might also be a pigment mutant, but it would be classified only as a morphological mutant.

Mycelial transfers from each of the colonies in the three viable mutant groups were next made to agar slants of minimal medium to detect any deficiencies in ability to synthesize essential nutrilites. More than a thousand normal-appearing colonies grown from irradiated spores were also tested on minimal medium, but none of these normal-appearing colonies ever proved completely deficient in any vital synthetic ability. Among the visible mutants a small fraction failed to grow on the minimal medium or grew very poorly. These mutants were apparently deficient in the ability to synthesize essential nutrilites. The particular nutritional requirements of these auxotrophic mutants were then determined by the standard procedures used in Neurospora research (BEADLE 1945) of testing mycelial transplants on various supplemented media. Although liquid media may be used in such tests, solid media are better for revealing partial reductions in ability to synthesize given nutrilites. It is apparent that this selection procedure restricts the auxotrophic mutants recovered to those which are visibly different on complete medium, to those requiring only heat-stable diffusible substances present in yeast or malt extract, and to those that are not inhibited by any of the components of the complete medium in the concentrations encountered there.

Identification of mutants

All the mutants which were found to affect the synthesis of the same metabolite were further tested for genetic identity. If after segregation from a cross

to the wild type an obvious difference in appearance still existed between mutants, they were then classified as different and no further genetic tests were generally made, even though closely linked mutations would thus be overlooked. Closely linked multiple mutations, however, must be very rare. A few mutants with apparently identical morphological and nutritional characteristics were crossed. If no wild type progeny segregated among at least 100 segregants, then the mutants were classified as identical and were regarded as independent occurrences of the same mutation. A further test of identity, occasionally used, involved measurements of rates of reversion to wild type. By this technique even very similar alleles might be distinguished, although variations in the stabilizing effect of associated genes might be misconstrued as a difference in the stability of the alleles themselves. Nevertheless, alleles with widely different back mutation rates which remain constant in association with various genic backgrounds may reasonably be regarded as representing genic configurations of different stabilities.

Tests for allelism in Glomerella are easily performed with nutritional mutants by plating out ascospores from a sexual cross in a minimal medium. Only nutritionally independent segregants (prototrophs) will grow on such a medium and the presence of such colonies establishes the non-allelic nature of the genes concerned. Furthermore, a simple elaboration of this technique should be suitable for determining linkage relationships among genes controlling biochemical syntheses. The proportions of the expected kinds of progeny among the ascospores resulting from a cross between two nutritional mutants may be determined by counting the ascospore-produced colonies which grow on minimal medium, on each of the two kinds of single-supplement medium and on the double-supplement medium. Only nutritionally wild type colonies (prototrophs) will grow on the minimal medium; prototrophs and a single mutant type will grow on each of the single-supplement media, and all types will grow on the double-supplement medium. By appropriate subtractions one may obtain the number of single mutants and double mutants and thus calculate linkage relationships from extensive data easily obtained by simply counting colonies on Petri dishes.

Sources of radiation

In the induction of mutants by radiation a source of X-rays was provided by a Westinghouse industrial X-ray unit with a Machlett thermax tube operated at 120 kilovolts and 8 milliamperes. In the irradiations reported here, a 1 ml suspension of conidia was placed in a small beaker in the X-ray beam 9 cm from the target. At this distance the dosage was 1670 r per minute. Exposures varying between 6 minutes and 120 minutes were employed, thus giving a range of X-ray dosage from approximately 10,000 r to 200,000 r.

Fast neutron irradiation was provided by the atomic pile at Oak Ridge, Tennessee. Dosage of fast neutrons as measured by a Victoreen thimble chamber varied between 27,670 rep and 133,000 rep. There was also a 5 to 10 percent gamma ray contamination not included in the listed neutron doses. The

entire culture tube containing a sporulated mycelium was exposed to the neutron bombardment in contrast to the use of a suspension of spores in distilled water in the case of X-radiation and ultraviolet irradiation.

The ultraviolet irradiation was produced by a Westinghouse WL 782 sterilamp which emits over 80 percent of the radiant energy in the region of 2537 Å. Spore suspensions were placed in an open shallow Petri dish (or occasionally in a quartz flask) on a rotating turntable 9 cm from the bottom of the sterilamp. At this distance the intensity of the radiation was 4,770 ergs/cm^2/sec, and the time of exposure was varied between 60 seconds and 25 minutes.

RESULTS

Mutation related to dosage and type of radiation

The data of this investigation are largely presented in table 1. It is evident from an examination of this table that the relative proportions of the various types of mutants do not vary with dosage in any consistent fashion for any of the three radiations used. Although there is considerable variability among the small numbers involved, the variation is not consistent and is probably of no significance. One may conclude, therefore, that no conspicuous qualitative differences in mutagenic effect are associated with a particular quantity of any type of radiation.

There are of course different quantitative effects at different doses. In general, the mutation rate rises linearly with increasing doses of fast neutrons or X-rays. By contrast, however, the mutagenic effect of ultraviolet radiation reaches a peak at 120 seconds and declines thereafter with increasing dosage.

Since dosage has no qualitative significance, the effects at different doses may be added together to provide data with which to compare the three types of radiation. These subtotals (table 1) show that the three major classes of mutants—morphological, pigment, and retarded—occur in similar proportions with each type of radiation. The morphologicals are the most common type after X-radiation and ultraviolet radiation, whereas neutron bombardment induces relatively more pigment mutants. However, these differences are of very doubtful significance, particularly in view of the subjective factors involved in their evaluation.

The nutritional mutants, it may be noted in table 1, have been divided into two groups on the basis of the magnitude of their induced nutritional requirement. Those with an absolute requirement for some nutrilite produce essentially no growth on a minimal medium; this group corresponds to the usual class of "biochemical mutants" among fungi, for example those in Neurospora. The larger group of nutritional mutants are those with only a partial requirement for additional nutrilites. This group will grow indefinitely on a minimal medium although at a conspicuously reduced rate as compared to their growth on appropriately supplemented media. The division between these two groups is rather arbitrary, since among the visible mutants of Glomerella there is almost a continuous gradation in ability to grow on a minimal medium

TABLE 1

Radiation-induced mutants related to type and dosage of radiation.

Dose	Colonies examined	Viable mutants		Types of viable mutants			Nutritional mutants	
		Number	Percent	Morphological	Pigment	Retarded growth	Absolute	Partial
				Ultraviolet				
60 sec.*	629	122	19	37	43	42	6	19
120	624	256	41	105	82	69	29	40
180	717	183	26	94	47	42	19	32
240	468	74	16	28	26	20	5	11
300	78	13	17	2	6	5	0	1
1,500	84	5	6	2	1	2	0	1
Subtotals	2600	653	25	268	205	180	59	104
% of viable mutants	....	....	....	41%	31%	28%	9%	16%
				Neutron				
27,670 rep	629	68	11	14	40	14	3	7
54,000	655	95	15	28	55	12	1	14
67,650	611	139	23	57	44	38	9	17
85,200	559	193	35	48	63	82	8	14
93,000	236	54	23	16	33	5	2	5
100,000	817	333	41	106	91	136	11	26
133,000	132	69	52	30	14	25	7	3
Subtotals	3639	951	26	299	340	312	41	86
% of viable mutants	....	....	....	31%	36%	33%	4%	9%

TABLE 1 (*Continued*)

Dose	Colonies examined	Viable mutants		Types of viable mutants			Nutritional mutants	
		Number	Percent	Morphological	Pigment	Retarded growth	Absolute	Partial
				X-ray				
10,000 r	332	6	2	2	2	2	0	0
20,000	350	20	6	5	12	3	0	3
30,000	333	33	10	10	14	9	2	2
40,000	302	33	11	13	10	10	0	6
50,000	447	52	12	15	23	14	1	3
60,000	276	42	15	15	10	17	1	5
80,000	532	144	27	52	52	40	7	16
100,000	443	144	32	57	29	58	9	16
125,000	407	130	32	56	43	31	9	15
150,000	537	163	30	72	52	39	12	17
160,000	181	71	39	37	14	20	2	9
175,000	318	118	37	51	39	28	10	13
200,000	142	60	42	18	19	23	2	6
Subtotals	4600	1016	22	403	319	294	55	111
% of viable mutants	....	....	....	40%	31%	29%	5%	11%
Grand Total	10839	2620	24	970	864	786	155	301
% of viable mutants	....	....	....	37%	33%	30%	6%	11%

* 4770 ergs/cm²/sec.

from those with normal growth to those which fail to grow at all. Nevertheless, the great majority of the visible mutants when grown on minimal medium readily fall into one of three categories: 1) those completely failing to grow, 2) those with conspicuously reduced growth, and 3) the vast majority which grow normally.

The results of the tests for growth on a minimal medium are remarkably uniform for the three types of radiation. In each instance the proportion of mutants with partial to those with absolute requirements is about 2:1. Although these relative proportions are constant, the absolute effectiveness of ultraviolet light in inducing nutritional deficiencies appears to be noticeably greater than that of the ionizing radiations. The percentage of ultraviolet-induced visible mutants with nutritional deficiencies (table 1) is 25 percent as compared to 16 percent for X-rays and 13 percent for neutrons. The comparable percentages for those mutants with absolute requirements are 9 percent for ultraviolet, 5 percent for X-rays, and 4 percent for neutron bombardment. Although these percentage differences may suggest qualitative differences between the three types of radiation, the total numbers of mutants involved are too small to be decisive.

Mutant stability related to type of inducing radiation

However, it should not be assumed that the mutagenic effects of these radiations are identical, for the capacity to revert to standard type is undoubtedly greater among the ultraviolet-induced mutants than it is among the mutants produced by ionizing radiations. By reference to table 2 it may be seen that

TABLE 2

Spontaneous reversion to wild type among Glomerella auxotrophs.

Inducing radiation	Number of auxotrophs	Number reverted
Neutron	41	7
X-ray	55	9
Ultraviolet	59	33
Total	155	49

the ultraviolet-induced mutants revert to standard type more than three times as frequently as do the other mutants. The listed reversions refer only to the number of mutant strains which have been observed to revert spontaneously in culture at least once. A few of these mutants have reverted frequently and several have been induced to revert under the action of ultraviolet radiation. Reversion to standard type in culture must be at least partly a function of the length of time the mutant has been retained in culture. If the duration in culture is considered the divergence between the ultraviolet-induced mutants and the X-ray and neutron-induced mutants becomes even greater, since the ultraviolet mutants were the last to be induced and therefore have had the least opportunity to revert. However, reversions to standard type, when they occur at all, usually occur early in the culture life of the mutant. Reversions to

standard type among Glomerella auxotrophs are easy to identify since reversion always involves a change in visible characteristics as well as in synthetic ability.

Glomerella auxotrophs

The specific nutritional requirements of each of 75 mutants with an absolute requirement have been determined, and the results are presented in table 3. These auxotrophic mutants are, fortuitously, about equally divided among the three types of radiation: 22 by X-radiation, 25 by ultraviolet, and 24 by neutrons. Four additional mutants (2 lysine, 1 histidine, 1 biotin) although listed

TABLE 3

Glomerella auxotrophic mutants.

Nutritional requirement	Number of mutants induced by			Total	Number of distinct phenotypes
	X-ray	Ultraviolet	Neutron		
Methionine	5	3	3	11	5
Arginine	1	4	4	9	3
Adenine	1	4	3	8	7
Lysine	1	3	4*	8	4
Isoleucine-valine	2	0	5	7	4
Tryptophane	1	2	3	6	6
Thiamin	3	2	0	5	5
Pyridoxine	3	2	0	5	5
Biotin	1	3	1*	5	5
Leucine	1	0	3	4	1
Nicotinamide	1	1	1	3	1
Cytosine	1	0	0	1	1
Inositol	0	1	0	1	1
Histidine	0	0	1*	1	1
Glutathione	1	0	0	1	1
Total	22	25	28	75	50

* The histidine, biotin, and two of the lysine mutants were induced by ionizing radiation from an atomic explosion.

in the neutron column were induced by ionizing radiation released by an atomic explosion and therefore the particular inducing radiation is unknown. It appears from table 3 that the three types of radiation induce the same kinds of auxotrophic mutants and in reasonably similar proportions. Additional data might, of course, reveal differences which have been obscured by the limited data so far accumulated.

Mutations affecting the synthesis of the same nutrilite may represent changes at the same locus or at different loci. Exhaustive genetic tests involving the crossing of each mutant to all of the other mutants with the same growth requirement would, of course, reveal any allelism among the mutants. Because of the time and labor involved, this method has not been extensively used in this investigation. However, other methods are available for recognizing genetically different mutants which grow on the same nutrilite. At least three classes of mutants—methionine, arginine, and adenine—may be subdivided by differ-

ent growth responses of the mutants to precursors of the listed requirement. The eleven methionine mutants may thus be divided into three groups: four mutants are unable to convert SO_3 to S_2O_3, four are unable to convert cysteine to homocysteine, and three are unable to convert homocysteine to methionine. Among the arginine auxotrophs four will also grow on ornithine or citrulline, but five require arginine. Among the eight adenine mutants, five will grow on hypoxanthine, and three will not. Guanine will satisfy the requirement of all the adenine mutants. None of the other groups of auxotrophs have been subdivided by differential response to precursor nutrilites.

In addition to distinguishing auxotrophs by differential response to precursor nutrilites, it is also frequently possible to distinguish auxotrophs with

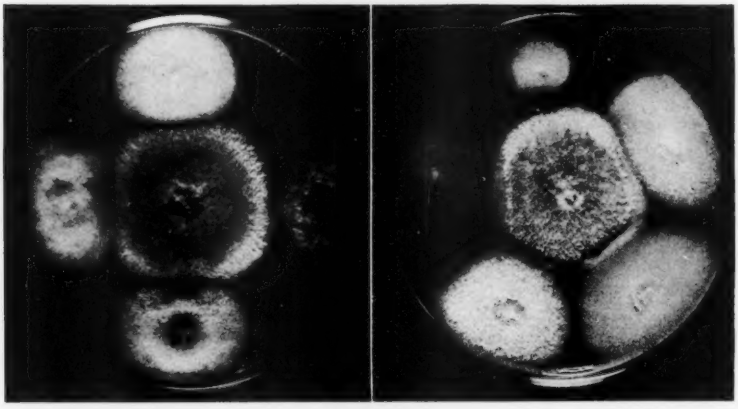

FIGURE 1.

Left. The colony in the center is standard type and the four peripheral colonies are thiamin mutants. Differences in color not shown by these photographs also distinguish these colonies. Cultures were grown for five days on a complete medium.

Right. The colony in the center is standard type and the five peripheral colonies are tryptophane mutants—the two colonies on the right possessing allelic genes for tryptophane synthesis.

apparently identical growth requirements by their visible differences in morphology or pigmentation (fig. 1). Such differences, developing under identical environmental conditions, imply genetic differences. When such differences in appearance persist after reisolation from a cross to the wild type, then it may reasonably be concluded that the auxotrophs are genetically different even though possessing the same growth requirements. Even mutant alleles may be distinguished by visible mycelial characteristics. Two of the tryptophane mutants which are apparently allelic (no prototrophs in more than 300 segregants between them) are nevertheless distinctly different in appearance. The distinction between these two alleles is further confirmed by their vastly different reversion rates to the standard type.

As judged by visible characters each of the five biotin, five pyridoxine, six

tryptophane (see fig. 1), and five thiamin mutants is different from all others, whereas the three nicotinamide mutants are alike and the four leucine mutants appear to be identical. The eight lysine mutants represent four visibly different types and the seven isoleucine-valine mutants are divided by appearance into four groups. Among the eight adenine mutants only two appear identical. Thus there may be seven distinct adenine mutants even though they are divisible into only two groups by their differential response to hypoxanthine. The arginine mutants were, as previously pointed out, divisible into two groups by their growth requirements. The four that grew on ornithine or citrulline are identical in appearance but different from the five that can only grow on arginine. One of the arginine-requiring mutants is visibly different from the remaining four. Growth tests subdivided the eleven methionine mutants into three groups, but on the basis of appearance there are at least five different types. The validity of visible characteristics as an index of auxotrophic genotype is in part demonstrated by the absence of any contradiction between appearance and growth response. Any two mutants with different growth responses are visibly different on complete medium. Thus of the 75 identified auxotrophs, at least fifty, probably, represent distinct gene changes (two of which are at the same locus). The remaining twenty-five mutants may prove to be independent repetitions of previously recorded gene changes although apparent identity of growth responses and appearance do not prove genetic identity.

The mutants with diminished synthetic ability (expressed as very restricted growth on minimal medium) were also tested on various supplemented media to determine their specific growth requirements. Unfortunately the great majority of these mutants appear to have complex requirements. They generally require vitamin rather than amino acid or nucleic acid supplementation for normal growth, and usually the vitamin requirement is not specific—a mixture of several vitamins having a decidedly more salutary effect than any one of them.

DISCUSSION

The results of this investigation support the conclusion that X-rays, fast neutrons, and ultraviolet radiation induce no qualitatively distinct types of mutants at any of the dosages employed. Moreover, the relative proportions of the types of mutants produced does not seem to be related to type or dosage of radiation. In general, these results correspond with those obtained in other investigations (TATUM 1950). However, the greater rate of reversion to wild type among ultraviolet-induced mutants implies that the physical or chemical changes induced in the gene by ultraviolet may frequently differ from the changes induced by the ionizing radiations even though the genetic effects on the physiology of the organism may be indistinguishable. The peculiar dosage effects of ultraviolet on mutation rate in Glomerella also confirm the results of HOLLAENDER *et al.* (1945) on Neurospora in demonstrating first a rise and then a decline of mutation rate with increasing dosage.

It should be emphasized that all of the auxotrophic mutants of Glomerella are also visibly different from the wild type. This sensitivity of the mycelium of Glomerella in visibly manifesting changes in synthetic ability is in marked contrast to Neurospora in which few biochemical mutants are noticeably different from the wild type (*cf.* SWANSON *et al.* 1949). The distinction drawn between morphological and biochemical mutants by SWANSON *et al.* could not apply to Glomerella.

The search for auxotrophs in Glomerella is rendered distinctly easier by their visibility, since only the visible mutants need be tested for ability to grow on minimal medium. A considerable concentration of desired mutants is therefore achieved among the tested colonies. One may reasonably expect to find between four and nine percent (depending on type of radiation used) of all the visible mutants to possess an induced absolute nutritional requirement.

The Glomerella mutants with only partial requirements represent a large group which is difficult to analyze. Since the nutritional requirements of these mutants are seldom simple, it seems reasonable to assume that the mutant gene is not primarily concerned with the synthesis of any simple "terminal" metabolite, but instead must affect some basic synthetic mechanism underlying a large sector of the metabolic activities of the cell. Such mutations do not abolish any vital syntheses since the mutants will grow indefinitely, albeit poorly, on a minimal medium. It may be instructive to compare these partial auxotrophs with the tyrosinase mutants (MARKERT 1950) previously described in Glomerella. The quantity of tyrosinase synthesized by Glomerella was shown to vary over a wide range and to be dependent upon the interaction of a large number of genes. Similarly the partial auxotrophs show quantitative variations in the ability to synthesize various simple nutrilites, and a large number of different genes again appear to affect the quantity synthesized by any strain of Glomerella. Furthermore, the diminished synthetic ability is not restricted to one nutrilite but more generally diminishes the synthesis of several apparently unrelated vitamins.

The class of mutants with diminished synthetic ability is also found in Neurospora, but these mutants have not received much attention. Many auxotrophs with absolute requirements have been identified in Neurospora, and since the selection procedures used in Neurospora research are similar to those used with Glomerella certain comparisons may be drawn between the auxotrophs found in the two fungi.

Comparative gene stabilities in Glomerella, Neurospora, and Salmonella

In Glomerella only two mutant types have occurred which have not previously been found in Neurospora. These are the biotin mutants and the glutathione mutant. Since wild type Neurospora requires biotin, no biotin mutants are obtainable, although a gene change at some locus in Neurospora might restore or create the capacity to synthesize biotin. No such change, however, has as yet been observed. The peptide mutant of Glomerella, having occurred only once, scarcely indicates any important difference between the two fungi.

More significant is the fact that the most common types of mutants found in Neurospora (TATUM 1950) have also been found in Glomerella (see table 3) and generally in similar relative proportions. The four most commonly-occurring mutants of Glomerella also are the four most commonly found in Neurospora. These parallel frequencies of the various types of mutants of Neurospora and Glomerella should not be surprising in view of the fact that the two fungi are related ascomycetes, belonging to the same order—Sphaeriales, but it is nevertheless interesting that the relative stabilities of genes controlling homologous syntheses in different organisms should not change greatly even after long periods of evolutionary separation.

That genes controlling homologous syntheses in distantly related organisms do not necessarily have comparable stabilities is illustrated by the data on auxotrophs of the bacterium *Salmonella typhimurium* (PLOUGH *et al.* 1950). The most common type of auxotrophs in Salmonella are again as in Glomerella and Neurospora those related to sulfur metabolism. However, histidine auxotrophs in Salmonella are the second most common type, and by contrast in Glomerella and Neurospora histidine mutants are very rare. An additional difference between Salmonella and the fungi lies in the auxotrophs with alternative amino acid requirements. These are relatively common in Salmonella, but rare in Glomerella and Neurospora.

The frequency with which any type of auxotroph occurs is of course dependent upon several factors: individual gene stability, number of genes involved in the synthesis, viability of the mutants, etc. However, the number of gene-controlled steps in the synthesis of a given nutrilite seems unlikely to vary greatly in two closely related organisms, and indeed, metabolic pathways have been found to be remarkably similar even in very different organisms. On the other hand, it is well established that alleles may have vastly different stabilities as, for instance, the allelic tryptophane mutants of Glomerella previously referred to.

Since there appears to be abundant opportunity for great variation in the stability of genes controlling homologous syntheses, the lack of such variation in Neurospora and Glomerella is rendered more impressive. A strong and similar selective advantage apparently is attached to the particular stabilities characterizing the genes which control homologous syntheses in Neurospora and Glomerella.

SUMMARY

1. The object of this investigation was to study the comparative mutagenic effect of X-rays, fast neutrons, and ultraviolet radiation on conidia of the fungus, Glomerella. No qualitative differences in mutagenic effect were found to be associated with any particular quantity of any of the three radiations used, nor did the kinds or relative proportions of mutants change with dose or type of radiation.

2. Reversion of auxotrophs to standard type occurred three times as frequently among ultraviolet-induced mutants as among those induced by ionizing radiations, thus suggesting that the physical-chemical changes induced in the

mutant gene by ultraviolet radiation are less stable than comparable changes induced by ionizing radiations.

3. In contrast to Neurospora, all Glomerella auxotrophs are visibly different from the standard type on a complete medium.

4. Among the visible mutants of Glomerella there is a continuous gradation in synthetic ability between those completely unable to grow on minimal medium and those with normal growth on minimal medium. The majority of nutritionally deficient mutants of Glomerella will grow indefinitely, although very poorly, on minimal medium.

5. The relative frequencies of various auxotrophs of Glomerella, Neurospora, and Salmonella are compared.

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LITERATURE CITED

BEADLE, G. W., 1945 Biochemical genetics. Chem. Rev. **37**: 15–96.

HOLLAENDER, ALEXANDER, EVA R. SANSOME, E. ZIMMER, and M. DEMEREC, 1945. Quantitative irradiation experiments with Neurospora crassa. II. Ultraviolet irradiation. Amer. J. Bot. **32**: 226–235.

MARKERT, C. L., 1949 Sexuality in the fungus, *Glomerella*. Amer. Nat. **83**: 227–231.

1950 The effects of genetic changes on tyrosinase activity in Glomerella. Genetics **35**: 60–75.

PLOUGH, H. H., HELEN N. YOUNG, and MADELON R. GRIMM, 1950 Penicillin-screened auxotrophic mutations in Salmonella typhimurium and their relation to x-ray dosage. J. Bact. **60**: 145–157.

SANSOME, EVA R., M. DEMEREC, and ALEXANDER HOLLAENDER, 1945 Quantitative irradiation experiments with Neurospora crassa. I. Experiments with X-rays. Amer. J. Bot. **32**: 218–226.

SWANSON, C. P., W. D. MCELROY, and HELMA MILLER, 1949 The effect of nitrogen mustard pretreatment on the ultra-violet-induced morphological and biochemical mutation rate. Proc. Nat. Acad. Sci. **35**: 513–518.

TATUM, E. L., 1950 Effects of radiation on fungi. J. Cell. Comp. Physiol. **35** (suppl. No. 1): 119–131.

WESTERGAARD, M., and H. K. MITCHELL, 1947 Neurospora. V. A. synthetic medium favoring sexual reproduction. Amer. J. Bot. **34**: 573–577.

WHEELER, H. E., 1950 Genetics of Glomerella. VIII. A genetic basis for the occurrence of minus mutants. Amer. J. Bot. **37**: 304–312.

WHEELER, H. E., and J. W. MCGAHEN, 1952. Genetics of Glomerella. X. Genes affecting sexual reproduction. Amer. J. Bot. **39**: 110–119.

THE TRANSMISSION OF MARKER GENES IN INTRASPECIFIC BACKCROSSES OF GOSSYPIUM HIRSUTUM L.

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ONE polyploid species, *Gossypium hirsutum* L., commonly called American Upland cotton, accounts for practically all the cotton produced in the United States. RICHMOND (1951) stated that "Reselection within varieties, and even within the progeny of varietal hybrids, over a period of many years inevitably has resulted in severe inbreeding and the elimination of many beneficial as well as deleterious genes which were present in the native stocks. Since American Upland varieties in the United States are all interrelated and probably descended from not more than a dozen original introductions, it is doubtful if future requirements of special fiber properties, disease, insect, and drought resistance, mechanical harvesting, and other specialized uses and properties can be met by the usual selection methods restricted to present cultivated varieties." Because of the need for greater genetic variability plant breeders are attempting to improve American Upland cotton by transferring to it genes from the tetraploid species, *G. barbadense* L., and from induced polyploids which are combinations of diploid species. The success of this program depends on an understanding of the differences in behavior of intraspecific and interspecific hybrid material.

A thorough review of interspecific cotton hybrids was given by STEPHENS (1950), who concluded that in addition to multiple gene substitution it is necessary to assume small structural differences between the chromosomes of the closely related genomes.

STEPHENS (1949) produced an F_1 hybrid between marked lines of *G. hirsutum* and *G. barbadense*. When the F_1 was backcrossed to each parent, there was considerable selective elimination of the donor parent genotype. The conclusion was that these results were peculiar to this interspecific hybrid, and reasons were given for believing that the chromosomes of the two species contained cryptic structural differences. The evidence for this conclusion was not completely critical, since the absence of selective elimination was not demonstrated in intraspecific backcrosses. It is possible that the selective elimination of the donor parent genotype was caused by the particular marker stock used or by the marker genes *per se*. Additional evidence would be furnished by a study of intraspecific backcrosses using similarly marked stocks of the one species, *G. hirsutum*. The purpose of this paper is to describe the transmission of marker genes in intraspecific backcrosses of certain stocks of *G. hirsutum*.

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METHODS

The *G. hirsutum* stocks used in this experiment were (1) SL 7-9, a multiple marker line carrying five dominant genes and one recessive gene, (2) Pubescent, (SIMPSON 1947), carrying contrasting alleles to those of SL 7-9 at the same six loci, and (3) Deltapine 14, a commercial variety carrying recessive genes at all six loci. The genetic constitution of each of these lines is shown in table 1.

A cross was made between SL 7-9 and Pubescent. The F_1 was backcrossed to each parent and crossed to the recessive tester stock, Deltapine 14. Although the cross of the $F_1 \times$ Deltapine 14 is not strictly a backcross, it will be considered as such for simplicity of discussion. The three backcross progenies were grown in the field in 1950 in a simple randomized block design. The randomized design was not necessary for the analysis of qualitative characters, but was used as a precaution against the loss or damage of plants in any particular spot in the experimental area. Each plant was scored for all characters that could be distinguished with certainty; the data from all replications were

TABLE 1

Genetic constitution of G. hirsutum marker lines.

SL 7-9		Pubescent		Deltapine 14	
R_1	Red plant body	r_1	Green plant body	r_1	Green plant body
R_2^{AF}	Weak spot	R_2^{AO}	Spotless	R_2^{AO}	Spotless
K	Brown lint	*k*	White lint	*k*	White lint
N	Naked seed	*n*	Not naked (fuzzy) seed	*n*	Not naked (fuzzy) seed
L^o	Narrow leaf	*l*	Broad leaf	*l*	Broad leaf
pb	Non-pubescent leaf	*Pb*	Pubescent leaf	*pb*	Non-pubescent leaf

pooled. Homozygous dominants could not be distinguished accurately from heterozygous individuals for petal spot, brown lint, naked seed, and pubescent leaf. In the backcross to SL 7-9, only the genes for plant body color, leaf shape, and leaf pubescence could be scored. In the backcross to Pubescent, all characters could be scored with ease except leaf pubescence. With the possible exception of petal spot, no difficulty was encountered in classifying any of the six characters in the backcross to Deltapine 14. The cross to Deltapine 14 was also used to test the linkage relationships between the pubescent gene and the five independent marker genes in SL 7-9.

RESULTS

The data from the backcrosses of F_1 SL 7-9 $\times$ Pubescent to SL 7-9, Pubescent, and Deltapine 14 are given in table 2. Chi-square values were calculated for each of the monofactorial segregations and, with one exception, none were significantly different from a 1:1 ratio. The segregation of the R_2^{AF}: R_2^{AO} genes in the $F_1 \times$ Deltapine 14 cross showed a deficiency of spot and an excess of spotless petals. The gene R_2^{AF} in the heterozygous condition often expresses itself so weakly that careful examination by a hand lens or a dissecting micro-

scope is necessary to detect a few colored cells in the petals. It is possible that in scoring the segregates of the $F_1 \times$ Deltapine 14 cross some weakly spotted flowers might have been scored as spotless when classified by the unaided eye in the field; however, the same alleles gave a good fit to a 1:1 ratio in the cross, $F_1 \times$ Pubescent. Why the R_2^{AF} gene would express itself more clearly in a cross with Pubescent than with Deltapine is not apparent from the data available.

STEPHENS (1949) reported a highly significant deficiency of the *K* type in the segregation of *K*:*k* in the backcross to *hirsutum* and a deficiency that almost reached significance in the backcross to *barbadense*. In the present work the segregation of *K*:*k* gave a good fit to a 1:1 ratio in crosses of $F_1 \times$ Pubescent and $F_1 \times$ Deltapine 14. This finding demonstrates again that the white lint gene *per se* has no selective advantage over its brown lint allele.

TABLE 2

Monofactorial segregation in backcrosses of intraspecific G. hirsutum hybrids. F_1 refers to SL 7-9 × Pubescent and alleles from the SL 7-9 stock are shown on left in each case.

	R_1	r_1	R_2^{AF}	R_2^{AO}	K	k	N	n	L^o	l	pb	Pb
$F_1 \times$ Pubescent	251	271	257	260	253	267	252	268	253	269	....	....
χ^2 (1:1)	.766		.017		.377		.492		.490		....	
P (1)	.30–.50		.80–.90		.50–.70		.30–.50		.30–.50			
$F_1 \times$ SL 7-9	249	256	....	....	....	....	....	....	241	264	254	251
χ^2 (1:1)	.097		....		....		....		1.048		.018	
P (1)	.70–.80								.30–.50		.80–.90	
$F_1 \times$ Deltapine 14	292	293	250	331	314	269	281	302	287	298	302	283
χ^2 (1:1)	.001		11.293**		3.473		.756		.207		.617	
P (1)	.98		<.01		.05–.10		.30–.50		.50–.70		.30–.50	

** = significance at .01 level of probability.

The monofactorial segregations of all the genes, except the questionable classification of petal spot, did not deviate significantly from a 1:1 ratio; however, the gene from the donor parent was deficient in all five classifications in the cross, $F_1 \times$ Pubescent, and in one of the three classifications of $F_1 \times$ SL 7-9. In the cross, $F_1 \times$ Deltapine 14, the gene from SL 7-9 was deficient in four out of six classes. Although the individual segregations were not significantly different from a 1:1 ratio, the cumulative effect of slight deficiencies in several genes might be significant. To investigate this possibility, the frequencies of SL 7-9 genes expected on the basis of random recombination were calculated and compared with the actual numbers observed. This was done by expanding the binomial $(\frac{1}{2} + \frac{1}{2})^n$, where *n* is the number of marker genes that could be scored with certainty in each cross. The gene for petal spot was omitted from the cumulative totals since the classification was not considered completely reliable. In table 3 the actual numbers obtained are compared with the numbers expected. Chi-square values calculated from these data show that in each backcross the deviations from the expected on the basis of random recombinations are not great enough to be significant.

TABLE 3

Frequency of plants carrying various numbers of SL 7-9 marker genes in crosses of (a) F_1 × Pubescent, (b) F_1 × SL 7-9, and (c) F_1 × Deltapine 14 where the F_1 was SL 7-9 × Pubescent.

No. of SL 7-9 markers	5	4	3	2	1	0	Total	
(a) F_1 × Pubescent								
Expected $(\frac{1}{2} + \frac{1}{2})^4$	....	32.375	129.500	194.250	129.500	32.375	518	
Actual	....	24	138	174	146	36	518	
χ^2	....	2.167	0.558	2.111	2.102	0.406	7.344	(P(4) = .10–.20)
(b) F_1 × SL 7-9								
Expected $(\frac{1}{2} + \frac{1}{2})^3$	....	....	62.5	187.5	187.5	62.5	500	
Actual	....	....	61	178	197	64	500	
χ^2	....	....	0.036	0.481	0.481	0.036	1.034	(P(3) = .50–.95)
(c) F_1 × Deltapine 14								
Expected $(\frac{1}{2} + \frac{1}{2})^5$	18.125	90.625	181.250	181.250	90.625	18.125	580	
Actual	10	100	188	181	82	19	580	
χ^2	3.642	0.970	0.251	0.000	0.821	0.042	5.726	(P(5) = .30–.50)

TABLE 4

Segregation of pubescence in linkage tests with five independent marker genes in SL 7-9 using the cross (SL 7-9 × Pubescent) × Deltapine 14.

Pubescence with:	Frequency of genotypes				Total	χ^2 1:1:1:1	P(3)
Plant body color	R_1 Pb 144	R_1 pb 148	r_1 Pb 139	r_1 pb 154	585	0.83	.80-.90
Lint color	K Pb 155	K pb 159	k Pb 127	k pb 142	583	4.30	.20-.30
Seed cover	N Pb 142	N pb 134	n Pb 140	n pb 159	575	2.39	.30-.50
Leaf shape	L^o Pb 142	L^o pb 145	l Pb 141	l pb 157	585	1.11	.70-.80
Petal spot	R_2AF Pb 122	R_2AF pb 128	R_2AO Pb 158	R_2AO pb 172	580	11.83**	.001-.01
R_2AF *vs.* R_2AO	250		330			11.03**	.01-.02
Pb *vs.* pb	280		300			0.69	.80-.90
Linkage	294		286			0.11	.99-1.00

** = significance at .01 level of probability.

The linkage relationship of the Pubescent gene, *Pb*, had not previously been tested with the marker genes in SL 7-9. As shown in table 4, pubescence was found to be independent of the five other genes. The significant Chi-square for the segregation of petal spot and pubescence was due to the excess of spotless over spotted petals and not to any linkage relationship between the two genes.

DISCUSSION

In intraspecific backcrosses involving certain marked stocks of *G. hirsutum*, the marker genes were recovered as expected on the basis of random recombination. Each allele and combination of alleles proved to be equally viable both in gametes and in zygotes. The deficiency of the petal spot, R_2^{AF}, allele in the backcross to Deltapine 14 was considered to be a matter of reduced expressivity rather than an actual elimination of the gene.

SL 7-9, Pubescent, and Deltapine 14 vary in several quantitative characters and must differ by many genes other than the six qualitative ones with which this study is concerned, but no genic or structural differences interfered with the recovery of the marker genes in the backcross progenies. In view of the regular behavior of the intraspecific hybrids studied in this experiment, it is reasonable to infer that neither the marker genes *per se* nor the particular *G. hirsutum* line used were responsible for the disturbed ratios found in STEPHENS' (1949) material. The selective elimination of the donor parent genotype in interspecific backcrosses must have been caused by genic or structural differences between the " homologous " chromosomes of the two species, *G. barbadense* and *G. hirsutum*.

The Pubescent gene, *Pb*, was independent of five qualitative genes used in the study.

LITERATURE CITED

RICHMOND, T. R., 1951 Procedures and methods of cotton breeding with special reference to American cultivated species. Advances in Genetics Vol. IV. Academic Press Inc., New York.

SIMPSON, D. M., 1947 Fuzzy leaf in cotton and its association with short lint. J. Hered. **38**: 153–156.

STEPHENS, S. G., 1949 The cytogenetics of speciation in *Gossypium*. I. Selective elimination of the donor parent genotype in interspecific backcrosses. Genetics **34**: 627–637.

1950 The internal mechanism of speciation in *Gossypium*. Bot. Rev. **16**: 115–149.

DIFFERENCES IN SPECIFICITY OF THE ANTIGENIC PRODUCTS OF A SERIES OF ALLELES IN THE CHICKEN[1]

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THE ability of normal bovine serum to agglutinate differentially the erythrocytes of chickens was first reported by LANDSTEINER and LEVINE (1932). Later, using an inbred strain of Rhode Island Red chickens, OLSON (1943) concluded that these differences in reactivity were due to a single pair of allelic genes. He proposed that one allele produced a strongly reactive agglutinogen while the other had little or no antigenic effect. A dosage effect was displayed in that the cells of birds homozygous for the allele effecting the antigen were more reactive and had greater absorptive power than the cells of heterozygotes.

The purpose of the present study was to investigate further the inheritance of the agglutinability of chicken erythrocytes with normal bovine serum and to determine the genetic relationships of such agglutinogens with those then known to exist in the chicken (BRILES, MCGIBBON and IRWIN 1950).

MATERIALS AND METHODS

The chickens used in this study were from a group maintained by the Wisconsin Agricultural Experiment Station for the study of cellular antigens. They were second and third generation crosses of birds derived from Rhode Island Reds, Single Comb White Leghorns and Barred Plymouth Rocks.

Blood was taken from the chickens by making a small cut in a wing vein and allowing the blood to run into tubes containing an isotonic solution of sodium citrate (2.00 percent) and sodium chloride (0.42 percent). The cells were washed three times with saline solution (0.75 percent sodium chloride) before they were tested. The bovine sera were obtained from blood taken from the jugular vein. These sera and the chicken isoimmune reagents employed in this study were stored at -18 to -23°C.

The normal bovine sera were inactivated for 20 minutes at 56°C in a water bath before they were used in testing cells for agglutination. Three different bovine sera were tested in serial dilutions (1:1, 1:2, 1:4, 1:8, etc.) against erythrocytes of the various chickens. It was found that the sera were alike

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except for minor differences in titer. One of the sera was arbitrarily selected for use in this study; however, as a check, erythrocytes from various birds were occasionally tested with one of the other two sera. For the most part, the erythrocytes of each chicken were tested for agglutination with the selected bovine serum at two dilutions, 1:2 and 1:8, which were found to give the most effective differentiation of the types of cells.

Tests for agglutination were performed in small test tubes by adding one drop (0.05 ml) of a two percent cell suspension to two drops of diluted cattle serum. This mixture was shaken at intervals of 15 minutes. After 45 minutes at room temperature the tubes were observed macroscopically for agglutination. A second observation was made after a total incubation period of two hours.

The erythrocytes of the chickens used in this study were also tested with reagents specific for the A and B series of agglutinogens recently discovered in the fowl. The preparation of these reagents and the genetic classification of the genes producing these agglutinogens have been presented in detail by BRILES, MCGIBBON and IRWIN (1950). Of the nine alleles of the *A* locus (A^{23}, A^{123}, A^{1236}, A^{2346}, A^{23456}, A^{3456}, A^{237}, A^{8} and a), four were present in the families used in this study; viz., A^{23}, A^{23456}, A^{237} and A^{8}. Of the five alleles of the *B* locus (B^{15}, B^{25}, B^{3}, B^{4} and b) three were present in the birds under study—B^{15}, B^{3} and b. In each series the locus is designated by an italicized capital letter. The digits in the superscripts following the letters indicate the ability of the antigenic substance determined by the respective alleles to react with reagents having presumably homologous components. The letters a and b indicate alleles for which no antigenic effects have been detected. In general, reagents used to detect each antigen of the A and B groups are prepared by absorbing isoimmune sera with the red cells of selected chickens until only components specific for particular antigenic factors remain. The resulting reagents are designated by capital letters followed by digits; the capital letter indicates the locus of the allele producing the homologous antigen or antigenic substance and the digit or digits represent the antibody fraction or fractions present in the reagents. Thus, the four *A* alleles (A^{23}, A^{23456}, A^{237} and A^{8}) possessed by the birds used in this study were identified by the reagents A2, A3, A456, A7 and A8. The antigen determined by each allele is given a subscript designation corresponding to the superscript designation of the causative gene. Thus, the antigens produced by the *A* alleles above are designated A_{23}, A_{23456}, A_{237} and A_{8}, respectively.

The agglutination tests with the A and B reagents were performed in the same manner as with normal cattle serum, except that the first reading was made after incubation for one and one-half hours at room temperature and confirmatory readings were made following overnight incubation in the refrigerator.

EXPERIMENTAL RESULTS

Upon testing adult chickens from several families it was found that the birds could be divided into three general classes on the basis of the agglutination of

their erythrocytes with unabsorbed normal bovine serum and on their capacity to absorb bovine serum. The finding of these three classes is in agreement with the earlier work of OLSON (1943). These three classes will be referred to as "weak," "moderate" or "strong" reactors. The weak reactor class includes all birds whose cells either failed to agglutinate when tested with unabsorbed normal bovine serum at a 1:2 dilution, or else reacted only weakly at that dilution; none of these weakly reactive cells were agglutinated at a 1:4 dilution. The cells of the strong reactors were definitely agglutinated by a 1:16 dilution and to some extent by a 1:32 dilution of normal bovine serum. The moderate reactors were between these two extremes; the cells of some of these birds reacted strongly at a 1:2 dilution but only slightly at a 1:4 dilution, while those of others gave a fair reaction at a dilution of 1:8 or even 1:16. These classifications were made on the basis of readings made after the serum-cell mixtures had been incubated at 35°C for two hours. If tests were held longer than two hours, the difference between the moderate and strong reactors was less apparent as both tended to become completely agglutinated. Thus, the main difference between the moderate and strong reactors appeared to be in their relative rates of reaction.

To check further the above classification, normal bovine serum was absorbed separately with the three types of cells. A given quantity of the serum was diluted with an equal part of saline and placed in a water bath at 56°C for 20 minutes before the absorptions were made. The number of absorptions and the quantities of the three kinds of cells used to exhaust the serum of "homologous" agglutinins were different for each of the three kinds of cells. The erythrocytes from weak reactors were employed in a ratio of one part of cells to one part of the diluted serum. The cells were divided into two equal portions. The serum was mixed with one portion of the cells and allowed to incubate, with frequent agitation, at room temperature for 45 minutes; the serum was drawn off following centrifugation, mixed with the second portion of cells, and the mixture was allowed to stand at room temperature for one hour. After this second absorption the supernatant was tested for reactivity with each of the three types of cells. It was observed that the cells of weak reactors failed to react with the absorbed fluid while the cells of the moderate and strong reactors were readily agglutinated. A few absorptions of bovine serum were made employing the cells of weak reactors in a ratio of two to three parts of cells to one part of serum. It was found that the degree of reaction of the cells of moderate and strong reactors to bovine sera so absorbed was not significantly reduced. Thus, it appeared that absorbing bovine serum with the cells of weak reactors did not remove to any appreciable extent the agglutinins for moderate or strong reactors.

To effect complete absorption with the cells of moderate reactors, it was usually necessary to use two volumes of cells to one volume of diluted serum and to divide the cells equally into three successive absorptions. These absorptions were carried out as described above, except that the third absorption was stored overnight in the refrigerator after it had been incubated at room temperature for one hour. Test-fluids thus prepared were then tested with the

cells of several birds representative of the three types of reactors. These tests demonstrated that the antibodies capable of agglutinating the cells of weak reactors had been removed, but agglutinins for the cells of strong reactors still remained in serum exhausted of agglutinins for moderate reactors. The moderate reactors all had similar but not duplicate absorptive capacities, especially on the basis of readings made after the tests had been stored overnight in the refrigerator, then allowed to stand one hour at room temperature (approximately 26°). Weak reactions for certain of the moderate group thus observed indicated that the moderate reactors differed in their affinities for bovine serum. Highly specific test-fluids which might have been used to detect these differences were not obtained although differential absorptions were attempted. The failure to obtain such test-fluids was possibly due to the rather broad specificity of antibodies in bovine serum exhibited towards the cells of the moderate and strong reactors.

When the cells of the strong reactors were used in absorbing bovine serum, three volumes of cells were used to absorb one volume of serum dilution. The absorption of agglutinins was more likely to be complete if the cells were divided into four equal parts and used in four successive absorptions—three at room temperature for three-fourths to one hour each and one, usually the last, for about 18 hours in the refrigerator in addition to one hour at room temperature. The cells of the strong reactors removed antibodies for themselves and also for the moderate and weak reactors.

In general, the above results confirm the finding of OLSON (1943) that there were three reactive classes into which the cells of various chickens could be grouped on the basis of their reactions to bovine serum. However, in the present study the somewhat variable results obtained following absorption with the cells of different moderate reactors suggested that the moderate reactors were not all alike with respect to their serological specificity.

In the hope of obtaining highly specific reagents capable of identifying the antigenic differences not clearly revealed by absorbed bovine serum, it was decided to attempt to produce test-fluids from isoimmune sera. Several immunizations were made between chickens whose red cells differed in reactivity to normal cattle serum. The method used in analyzing the resulting antisera has been reported in a previous paper (BRILES, MCGIBBON and IRWIN 1950). Analysis by this method showed that the isoagglutinins produced in such immunizations were of two kinds. One was found to be reactive with an agglutinogen which was also reactive with previously prepared reagents A2 and A3; this new agglutinin was assigned the symbol A7 and the agglutinogen was designated A_{237}. The second isoagglutinin was reactive with an agglutinogen not reactive with any of the previously prepared A or B reagents. However, tests for genetic segregation showed that this new agglutinogen was determined by a gene belonging to the *A* series of alleles. This agglutinogen and the reagent used to identify it were assigned the symbols A_8 and A8, respectively.

The identification of these two new agglutinogens, A_{237} and A_8, made it possible to mate birds of appropriate blood types so that families could be

produced in which the genotype at the *A* locus could be completely determined by direct agglutination test. On the basis of their reaction to the isoimmune reagents used to detect the A series of alleles, the three general classes of chicken cells detected by testing with bovine serum were found to fall into ten genotypic classes; the weak reactors were found to be of three genotypic classes, the moderate reactors fell into four classes, and the strong reactors were found to consist of three classes.

The family given in table 1 is from the mating of parents (both classified as moderate reactors to bovine serum) which possessed no allele in common

TABLE 1

*Agglutination of the red blood cells of members of family R426 × R450 with various dilutions of normal bovine serum.**

Members of family		Serum dilutions 1:2	1:4	1:8	1:16	Type of cell	Genotype at *A* locus	Genotype at *B* locus
Sire	R426	+++	+++	++	0	M**	A^{23456}/A^{8}	B^{15}/B^{3}
Dam	R450	++++	+++	0	0	M	A^{237}/A^{23}	b/b
F_1	C610	0	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C612	+++	++	++	+	M	A^{23456}/A^{23}	b/B^{3}
	C613	++++	++++	++++	++	S	A^{23}/A^{8}	b/B^{3}
	C614	++++	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C615	++++	+++	++	0	M	A^{237}/A^{8}	B^{15}/b
	C616	++++	+++	++	+	M	A^{23456}/A^{23}	b/B^{3}
	C617	++++	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C618	±	0	0	0	W	A^{23456}/A^{237}	b/B^{3}
	C619	±	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C620	++++	++++	++++	++++	S	A^{23}/A^{8}	b/B^{3}
	C622	+	0	0	0	W	A^{23456}/A^{237}	b/B^{3}
	C623	+++	++	±	0	M	A^{237}/A^{8}	B^{15}/b
	C624	±	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C635	+++	+++	+	0	M	A^{237}/A^{8}	b/B^{3}
	C2043	++++	++++	++++	++++	S	A^{23}/A^{8}	b/B^{3}
	C2649	++++	+++	++	+	M	A^{23456}/A^{23}	b/B^{3}
	C3351	±	0	0	0	W	A^{23456}/A^{237}	B^{15}/b
	C3352	++++	++	0	0	M	A^{237}/A^{8}	B^{15}/b
	C3353	±	0	0	0	W	A^{23456}/A^{237}	b/B^{3}

*Readings made after two hours of incubation.

**The letters W, M and S indicate "weak," "moderate" and "strong" reactors, respectively.

Symbols: 0 = no agglutination; ± = very weak agglutination; +, ++, +++ and ++++ indicate increasing degrees of agglutination.

at either the *A* or *B* locus. The genotypes of the nineteen offspring of this mating could be directly determined by test with appropriate A and B reagents. The genotype of the sire, R426, was $A^{23456}/A^{8}\ B^{15}/B^{3}$ while that of the dam, R450, was $A^{237}/A^{23}\ b/b$. As had been found in other matings between moderate reactors, their offspring were classified as strong, moderate or weak reactors with bovine serum. It was also noticed (table 1) that all offspring classified as strong reactors—C613, C614, C617, C620 and C2043—were of the genotype A^{23}/A^{8}. All offspring classified as moderate reactors—C612, C615, C616, C623, C635, C2649 and C3352—possessed only one of the two alleles comprising the genotype of the strong reactors, A^{23} or A^{8}, in combina-

tion with A^{23456} or A^{237}, respectively (*i.e.*, A^{23456}/A^{23} or A^{237}/A^{8}). The offspring classified as weak reactors—C610, C618, C619, C622, C624, C3351 and C3353—were of the genotype A^{23456}/A^{237}. Since the alleles A^{23} and A^{8}, together or in combination with either A^{237} or A^{23456}, were present in the genotype of all offspring whose red cells were agglutinated very strongly by the bovine serum, it was tentatively concluded that A^{23} and A^{8} were primarily responsible for the ability of the cells of certain members of this family to react with normal bovine serum. The antigens determined by the alleles of the *B* locus did not appear to be associated in any way with the agglutination of the cells of the members of this family by bovine serum. The B_3 antigen was distributed among the strong, moderate and weak reactors in a ratio of 3:4:3. The B_{15} antigen appeared among the strong, moderate and weak reactors in a ratio of 2:3:4. These ratios undoubtedly represent chance distributions.

TABLE 2

*Agglutination of the red blood cells of members of family A1543 × R635 with various dilutions of normal bovine serum.**

Members of family		Serum dilutions 1:2	1:4	1:8	Type of cell	Genotype at *A* locus	Genotype at *B* locus
Sire	A1543	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
Dam	R635	+++	0	0	M	A^{237}/A^{8}	B^{3}/b
F_1	C545	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C546	++	0	0	M	A^{237}/A^{23}	B^{15}/b
	C547	++++	++++	++++	S	A^{23}/A^{8}	B^{15}/b
	C548	+++	0	0	M	A^{237}/A^{23}	b/b
	C549	+++	0	0	M	A^{237}/A^{8}	B^{15}/B^{3}
	C550	++++	++++	++++	S	A^{23}/A^{8}	B^{3}/b
	C551	++++	++++	+++	S	A^{8}/A^{8}	B^{15}/B^{3}
	C991	+++	++	0	M	A^{237}/A^{23}	B^{3}/b
	C993	++++	++++	+++	S	A^{8}/A^{8}	B^{15}/b
	C994	++++	++	0	M	A^{237}/A^{23}	B^{15}/B^{3}
	C995	±	0	0	W?	A^{237}/A^{8}	B^{3}/b
	C2992	++++	+	0	M	A^{237}/A^{23}	B^{15}/b
	C2993	++	0	0	M	A^{237}/A^{8}	B^{3}/b

*Readings made after two hours of incubation.
For explanation of symbols, see table 1.

The reactivities to normal bovine serum of the red blood cells of 15 members of another family and the genotypes of the various members at the *A* and *B* loci are given in table 2. The sire of this family was classified as a strong reactor with bovine serum, and with respect to the *A* and *B* loci was of the genotype $A^{23}/A^{8}\ B^{15}/b$; the dam was classified as a moderate reactor and was of the genotype $A^{237}/A^{8}\ B^{3}/b$. The cells of the offspring of the genotype A^{23}/A^{8} (C545, C547 and C550) and A^{8}/A^{8} (C551 and C993) reacted strongly with bovine serum, even at a dilution of 1:8. The eleven remaining progeny possessed the allele A^{237} along with either A^{23} or A^{8}. The offspring of the genotype A^{237}/A^{23} (C546, C548, C991, C994 and C2992) were classed as moderate reactors since their cells clumped fairly strongly at a dilution of 1:2 of bovine serum, and three were reactive at a dilution of 1:4. Those of the

genotype A^{237}/A^{8} (C549, C995 and C2993) were reactive only at a dilution of 1:2 and the cells of one of the birds, C995, were so weakly reactive with bovine serum that on the basis of the single test performed they fell into the weak reactor class, those of the other two were placed in the moderate class. The weak reaction shown by the cells of this bird is probably accounted for by the somewhat variable nature of serological cross reactions; although, in general, the repeatability of the agglutination tests with normal bovine serum was found to be remarkably high throughout this study. As with the previous family, there was no apparent relationship between the reactions obtained with bovine serum and the genotypes at the *B* locus.

The results obtained with the above two families indicated that the alleles A^{23} and A^{8} produced agglutinogens strongly reactive with normal antibodies of bovine serum and that the alleles A^{23456} and A^{237} produced agglutinogens showing only slight affinity for normal bovine serum. Furthermore, a dosage

TABLE 3

Number of chickens of each genotype showing the various degrees of agglutination with normal bovine serum.

Genotype at *A* locus	Number of chickens tested	Type of cell	Agglutination of red cells with bovine serum									
			Serum dil. 1:2					Serum dil. 1:8				
			++++	+++	++	+*	0	++++	+++	++	+	0
A^{23}/A^{23}	1	S	1	0	0	0	0	1	0	0	0	0
A^{8}/A^{8}	2	S	2	0	0	0	0	0	2	0	0	0
A^{23}/A^{8}	16	S	16	0	0	0	0	8	7	1	0	0
A^{8}/A^{237}	16	M	8	4	3	1	0	0	0	0	1	15
A^{8}/A^{23456}	10	M	4	6	0	0	0	0	0	3	1	6
A^{23}/A^{237}	35	M	6	19	9	1	0	0	0	0	1	34
A^{23}/A^{23456}	22	M	11	11	0	0	0	0	1	2	2	17
A^{237}/A^{23456}	26	W	0	0	0	6	20	0	0	0	0	26
A^{237}/A^{237}	3	W	0	0	0	0	3	0	0	0	0	3
A^{23456}/A^{23456}	3	W	0	0	0	0	3	0	0	0	0	3

*Very weak reactions (±) are summarized above under the degree of reaction +.

effect was apparent in that the cells of birds possessing both A^{23} and A^{8} were more reactive than the cells of those possessing one of these alleles along with A^{23456} or A^{237}. To test this hypothesis, the parents and offspring of ten additional families were tested with appropriate A reagents and with 1:2 and 1:8 dilutions of bovine serum. A summary of these tests, including the data on the two families already presented, is given in table 3. In summarizing these data very weak reactions of the degree ± were included among the weak reactions indicated by the symbol +. Of the 134 birds included in this table, 19 belonged to the strong reactor class. These birds belonged to one of three genotypes—A^{23}/A^{23}, A^{8}/A^{8} or A^{23}/A^{8}. Thus, it appears as though A^{23} and A^{8} together are able to elicit approximately the same quantitative reaction with bovine serum as a double dose of either.

The reactions obtained at a 1:8 dilution of bovine serum served to differentiate readily the moderate from the strong reactors. In general, the moder-

ates reacted relatively weakly, if at all, while the strong reactors were usually strongly clumped at a 1:8 dilution. Even at a 1:2 dilution the moderate reactors usually failed to react with the strength characteristic of the strong reactors. Among the birds used in this study all moderate reactors possessed one of four genotypes—A^8/A^{237}, A^8/A^{23456}, A^{23}/A^{237} or A^{23}/A^{23456}. Each of these birds with moderately reactive cells thus possessed one and only one allele in common with those possessed by the strongly reactive birds, that is A^{23} or A^8. It is also apparent that each bird having moderately reactive cells possessed either the allele A^{23456} or A^{237}. The weak or non-reactive class of birds, except for bird C995 discussed earlier, belonged to one of three genotypes—A^{237}/A^{23456}, A^{237}/A^{237} or A^{23456}/A^{23456}.

DISCUSSION

The evidence presented indicates that the agglutinability of chicken erythrocytes by bovine serum was primarily, if not completely, determined in the population under study by four alleles of the *A* locus. Agglutination tests with isoimmune reagents clearly indicate that the antigenic product of each of the *A* alleles is biochemically distinct from that produced by the other members of the allelic series (BRILES, MCGIBBON and IRWIN 1950). Although the antigenic product of each of the four alleles has its own particular serological characteristics, the effects of their respective antigens on the agglutinability of erythrocytes by normal bovine serum appear to fall in one or the other of two categories in that they produce antigens having either very strong or very weak affinity for bovine serum. The antigenic products of the alleles A^{23} and A^8 are strongly reactive, while the antigens produced by the alleles A^{23456} and A^{237} are very weakly reactive, if at all, with bovine serum. (Although the assumption is made that these causative genes are members of an allelic series, the possibility of pseudo-allelism cannot be excluded.)

The over-all effect of the *A* locus on the agglutinability of chicken erythrocytes by bovine serum appears to be quantitative and determined by the total affinity contributed by the independently acting *A* alleles. For example, when antigen A_{23} was found in cells strongly reactive with bovine serum, it was present in *double dose* in the homozygote A^{23}/A^{23} or accompanied by the antigen A_8 in the heterozygote A^{23}/A^8; when antigen A_{23} was found in moderately reactive cells, it was accompanied by antigens A_{237} or A_{23456}, having negligible affinity for bovine serum. It is quite possible that the antigens A_{23} and A_8 are different in their affinity for bovine serum; it may be that their affinities are so similar that the quantitative agglutination test employed was inadequate to disclose definite antigenic differences, which are clearly demonstrable by qualitatively different isoimmune reagents.

Through the use of bovine serum alone, OLSON (1943) identified an antigenic locus to which he assigned the symbol *A*. On the basis of his work on bovine serum he concluded that at this locus probably two alleles were present which determined the antigenic affinity of chicken erythrocytes for bovine serum: *AA* being the genotype of the strong reactors, *Aa* the genotype of the

moderate reactors, and *aa* the genotype of those individuals whose cells showed negligible affinity for bovine serum. This locus may be identical with the first blood group locus (*A*) discovered independently by BRILES, MCGIBBON and IRWIN (1950) with isoimmune agglutinins. (The assigning of the symbol, *A*, to the locus was coincidental.) The latter work disclosed the existence of at least nine alleles at this locus. The affinity for bovine serum of the antigenic products of four of these alleles—A^{23}, A^{23456}, A^{237} and A^{8}—has been presented in this study. Unfortunately, time did not permit the testing of the affinity for bovine serum of the antigens of the remaining *A* alleles. It is possible that only two alleles determining antigenic affinity of the red cells for bovine serum were present in the inbred population investigated by OLSON; however, in view of the results reported in this paper, more than two alleles could have existed in the population studied by OLSON since the affinity for bovine serum of the antigenic products of different *A* alleles may be similar or perhaps identical.

Antigenic differences somewhat analogous to those demonstrated in the present study for chicken cells have been detected with normal bovine serum in human red blood cells by STORMONT (1949). He found that a normally occurring antibody, called anti-J, agglutinated human cells possessing the A antigen. This antibody is present only in the serum of certain cattle and presumably is due to a single gene. The bovine sera used in these studies agglutinated chicken red cells equally well irrespective of whether or not the antibody for J was present. Further, in recent tests of the sera of over 20 individual cattle, no difference in titer for chicken cells was found in cattle sera possessing the antibody for J as compared to those lacking the antibody (IRWIN, unpublished). However, it appears quite unlikely that the antibody in cattle sera reactive with human A_2 or O is related to the antibody specific for chicken cells.

Saline extracts from certain seeds have been shown by RENKONEN (1948) and BOYD and REGUERA (1949) to agglutinate human cells possessing certain antigens of the A–B–O blood group. For example, BOYD and REGUERA found that extracts from certain varieties of beans agglutinated only cells possessing antigen A. It appears reasonable to propose that further specificities in gene products may be demonstrated by naturally occurring antibodies.

SUMMARY

The agglutination of chicken erythrocytes by normal bovine serum appears to be determined by the alleles of the *A* series. The agglutinogens effected by these alleles were identified through the use of isoimmune reagents. Of the nine known alleles of the *A* group, four were utilized in this study. The agglutinogens produced by two of these alleles, A^{23} and A^{8}, reacted strongly with normal bovine serum. The antigenic products of the other two alleles, A^{237} and A^{23456}, reacted only very weakly, if at all, with bovine serum.

There was a distinct dosage effect of the reactive alleles A^{23} and A^{8}. The cells of individuals having a single dose of a reactive allele (for example, A^{23}/A^{237} or A^{8}/A^{237}) were only moderately reactive, while the cells of indi-

viduals possessing a *double dose* of the reactive alleles (A^{23}/A^{23}, A^{8}/A^{8} or A^{23}/A^{8}) were very strongly reactive.

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LITERATURE CITED

BOYD, W. C., and ROSE M. REGUERA, 1949 Hemagglutinating substances for human cells in various plants. J. Immunol. **62**: 333–339.

BRILES, W. E., W. H. MCGIBBON and M. R. IRWIN, 1950 On multiple alleles effecting cellular antigens in the chicken. Genetics **35**: 633–652.

LANDSTEINER, K., 1945 The Specificity of Serological Reactions. XIV + 310 pp. Cambridge: Harvard Univ. Press.

LANDSTEINER, K., and P. LEVINE, 1932 On individual differences in chicken blood. Proc. Soc. Exp. Biol. Med. **30**: 209–212.

OLSON, CARL, JR., 1943 The inheritance of an agglutinogen of the chicken erythrocyte. J. Immunol. **47**: 149–154.

RENKONEN, K. O., 1948 Studies on hemagglutinins present in seeds of some representatives of the family of Leguminoseae. Ann. Med. Exp. Biol. Fenn. **26**: 66–72.

STORMONT, C., 1949 Acquisition of the J substance by the bovine erythrocyte. Proc. Nat. Acad. Sci. **35**: 232–237.

A POLYHAPLOID OBTAINED FROM A HYBRID DERIVATIVE OF SORGHUM HALEPENSE × S. VULGARE VAR. SUDANENSE

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ALTHOUGH the origin of new and stable forms of plant species by means of chromosome doubling is well known to cytogeneticists, the reverse process of reduction in chromosome number in polyploids, such as the occurrence of haploids from diploids by parthenogenesis, is a rare phenomenon. This paper reports the occurrence of a diploid or polyhaploid plant in a population of tetraploid Sorghum of hybrid origin. This plant was a chance discovery; its sister plants were all tetraploids, and no other polyhaploids have yet been detected in the numerous cultures of tetraploid Sorghum grown at the University of California, Berkeley, where the present study was made. For these reasons, the discovery was of sufficient interest to warrant the cytological and morphological study of which the results are reported in this paper.

ORIGIN AND HISTORY OF THE POLYHAPLOID CULTURE

As has been reported by many authors (MYERS 1947), all forms of *Sorghum vulgare,* sens. lat., including var. *sudanense,* or Sudan Grass, are diploid, with 2n = 20 chromosomes; while *S. halepense,* or Johnson grass, is tetraploid with 2n = 40. This difference in chromosome number makes hybridization between the two species very difficult, and the triploid F_1 hybrids are sterile. On the other hand, hybrids between *S. halepense* and autotetraploid *S. vulgare* are easily made, and are fertile. The present material is descended from a tetraploid hybrid made by DR. L. F. RANDOLPH of Cornell University between artificially produced autotetraploid plants of Sudan Grass (*S. vulgare*) and the natural tetraploid species *S. halepense*. According to RANDOLPH (in litt. to G. L. STEBBINS, JR.), the female parent was a male sterile strain of *S. halepense* which was exposed to the pollen of tetraploid Sudan grass. The cultures which were studied for the present report were obtained from two different plants of the F_5 generation, grown in the garden of the Genetics Division, University of California, and descended from seeds of F_2 plants obtained from DR. RANDOLPH. One of these two F_6 cultures (No. 802) contained only tetraploid plants, while the other, no. 801, consisted entirely of diploids. The parent of culture no. 801 was a single F_5 plant, which probably arose from its tetraploid F_4 ancestor by means of parthenogenesis. Unfortunately, this F_5 plant had already been destroyed when the diploid nature of culture no. 801 was discovered. After this discovery, 30 plants each of the F_3 F_4, F_5, and F_6 generations were grown, but no diploids were found among

them. Evidently parthenogenesis is a rare occurrence in this material, and its detection was a lucky chance. Since culture no. 801 differed from all other diploids grown in our garden, and possessed certain morphological characteristics typical of its tetraploid relatives, it could not have resulted from accidental contamination of the parental culture.

EXPERIMENTAL PROCEDURE

The two strains, diploid and tetraploid, were compared under uniform conditions in two different experiments. The first was in the greenhouse, in a large wooden soil bin, which had a depth of about 50 cm. The seeds were planted in October, 1947, and the plants reached maturity in January and February, 1948. For the second, seedlings were started in the greenhouse in April, were planted in the field in May, and matured between August and September. Under greenhouse conditions, the seeds were planted in adjacent rows 1 foot apart, and the young seedlings thinned so that the plants which matured were spaced about 1 foot apart in the rows. For the field experiment, the seedlings were transplanted into rows 3 feet apart, and spaced the same distance apart in the rows. In this instance, there were planted in addition to the two F_6 cultures, tetraploid cultures of the F_3, F_4, and F_5 generations, obtained from seed harvested in previous years.

Somatic chromosomes were studied from root tips fixed in RANDOLPH'S " Craf " solution, sectioned and stained with iodine-gentian violet in the usual manner for this laboratory. Meiotic chromosomes were studied from aceto-carmine squash preparations of material previously fixed in a 3:1 absolute alcohol acetic acid solution. The percentage of good pollen was determined from mature anthers squashed and mounted in a solution of lactic phenol stained with cotton blue. At least 400 grains from 2 different flowers, were counted for each plant.

GENERAL MORPHOLOGY

During early stages of growth, both in the greenhouse and in the field, the polyhaploid and the natural diploid, Sudan 23, grew more slowly than the tetraploid hybrid derivatives of the F_5 and F_6 generations. At maturity, however, the tetraploid was not larger or more vigorous than the diploids. Table 1 shows the differences between the three strains in respect to the four most conspicuous morphological characteristics, and in pollen fertility. The polyhaploid is seen to be the tallest, the tetraploid intermediate, and Sudan 23 the shortest. On the other hand, the polyhaploid strain has significantly fewer tillers and broader leaves than the other two which do not differ significantly in these respects. The only reproductive character included was the nature of branching of the panicle. The length and number of nodes of the panicle was about the same in all three strains, but the polyhaploid bore a larger number of branches at the lower nodes than the other two. This difference was best estimated by counting the total number of branches per panicle.

One character not recorded in the table is the shape and color of the spikelets at maturity. In the tetraploid, they resemble those of *S. halepense* in being

relatively narrowly ovate, black, and shining. Sudan 23, however, has broader, yellowish spikelets which are somewhat duller. In this respect, the polyhaploid was indistinguishable from its tetraploid progenitors and differed from all other diploid strains of Sorghum known to us.

In perenniality, the polyhaploid strain was intermediate. Under conditions at Berkeley, Sudan 23 dies after maturing its seed, and never regenerates. The tetraploid hybrid derivatives, on the other hand, produce new shoots freely, and survive for several seasons. Most of the polyhaploid plants produced new shoots during the early winter after their first flowering season, but these were killed by an unusually heavy January frost, which also destroyed many of the tetraploids. After this time, no new regeneration took place.

TABLE 1

Comparison of certain morphological characters and pollen fertility in the polyhaploid, the tetraploid F_3, and diploid Sudan 23 under field conditions.

	Polyhaploid (822)	Tetraploid F_3 (821)	Diploid Sudan 23 (823)
Number of plants	28	30	30
Height of plant (cm)			
Range	152–254	86–218	116–187
Mean	207 ± 5.3	173 ± 5.6	149 ± 2.9
Number of tillers			
Range	22–119	43–196	39–134
Mean	65.0 ± 5.4	93.0 ± 11.0	83.3 ± 3.9
Width of leaves (mm)			
Range	17–40	13–48	14–27
Mean	27.2 ± 1.2	22.6 ± 1.4	21.0 ± 0.6
Number of panicle branches			
Range	21–56	17–54	14–34
Mean	34.8 ± 1.5	26.5 ± 1.2	24.4 ± 0.9
Pollen fertility (%)			
Range	71–88	7–70	70–96
Mean	75.6	41.4	84.0

The tetraploid hybrid derivatives were more variable in all characteristics than either of the diploid strains. This is shown in table 1 by the greater range of variation recorded for this type, and the larger size of the standard errors. In this respect, the polyhaploid strain was intermediate, though somewhat nearer to the tetraploid than to Sudan 23.

CYTOLOGY AND FERTILITY

Somatic divisions were normal in all three strains, under both greenhouse and field conditions. In meiosis, the diploid Sudan 23 was also normal in every respect, and as shown in table 1, its fertility was high and rather constant from plant to plant.

In the tetraploid hybrid derivatives, meiosis was essentially similar to that reported by HUSKINS and SMITH (1934) and GARBER (1944) for *S. halepense*. Bivalents and quadrivalents occurred in every sporocyte, while uni-

valents and trivalents were not infrequent. No association higher than quadrivalent was observed. The maximum number of quadrivalents per sporocyte was five, as found by GARBER in *S. halepense*. Later stages of meiosis were frequently irregular, and individual chromosomes were often eliminated from the microspores. This probably accounts for the high percentage of pollen abortion in many of the tetraploid plants.

The 20 chromosomes of the polyhaploid strain were usually paired normally as 10 bivalents, but occasional sporocytes contained 9 bivalents and 2 univalents (Figures 1, 2). Because of these univalents, a small percentage of irregularity was found in later stages of meiosis. This was enough to account for the slight reduction in pollen fertility of this strain, as compared to Sudan 23.

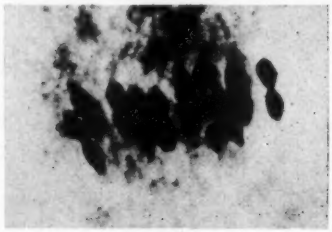

FIGURE 1.

FIGURE 2.

FIGURE 1.—A cell of the parthenogenetic diploid strain showing the 20 chromosomes paired normally into 10 bivalents. (× 2500.)

FIGURE 2.—Another cell of the parthenogenetic diploid strain showing 9 bivalent and 2 univalent chromosomes. (× 2500.)

DISCUSSION

The production of a fertile diploid from a tetraploid was first reported by HÅKANSSON (1924), who found a 14-chromosome plant in the progeny of the 28-chromosome autotetraploid *Oenothera gigantea*, a derivative of *O. Lamarckiana*. RANDOLPH and FISCHER (1939) found 23 parthenogenetic maternal diploids in a population of 17,165 individuals of autotetraploid maize. KATAYAMA (1935), who found haploid plants in progeny of the allopolyploid Aegilotriticum, observed that they were sterile. He concluded that the character of parthenogenetic derivatives with the reduced chromosome number could provide information as to whether the parental form was auto- or allopolyploid.

STEBBINS and KODANI (1944) found that a parthenogenetic haploid, or polyhaploid, produced from 72-chromosome guayule was weak, sterile, and had slightly irregular meiosis, and from this concluded that the parental type was at least partly allopolyploid. GERSTEL and MISHANEC (1950) reported the occurrence in this species of several such polyhaploids, some of which were relatively vigorous and fertile, indicating that 72-chromosome guayule is partly autopolyploid. ELLIOTT and WILSIE (1948) found a fertile polyhaploid in *Bromus inermis*, and assumed from this that the parental species is largely autopolyploid, but with some differences of homology among the parental genomes.

The meiotic behavior and fertility of the present polyhaploid strain are similar to that found in ELLIOTT and WILSIE'S polyhaploid of *Bromus inermis*. In the present instance, the progenitor of the polyhaploid was a descendant of an artificial hybrid between a synthetic autotetraploid and a second natural tetraploid which possesses many autotetraploid characteristics. Although both HUSKINS and SMITH (1934) and GARBER (1944) have suggested that the 20-chromosome types of the *Sorghum vulgare* complex might be ancient tetraploids they now behave essentially like diploids, and can be considered such for purposes of the present argument. On this assumption, the hybrid which gave rise to the present polyhaploid could be considered an autotetraploid only on the assumption that *S. halepense* is also an autotetraploid, and that its two genomes are completely homologous with the genome of *S. vulgare* var. *sudanense*. The considerable differences in external morphology between *S. vulgare* and *S. halepense* suggest that at least one of the two genomes of the latter species was derived from a species different from *S. vulgare*, a conclusion also reached by HUSKINS and SMITH. Furthermore, the slight meiotic irregularities found in the polyhaploid suggest that its two genomes are not completely homologous with each other, although they are very nearly so. That this strain contains some genes derived from *S. halepense* is evident from the appearance of its mature spikelets and its tendency toward the perennial habit. This whole situation could be explained on the basis of the following hypothesis. Assume that the genomic constitution of *S. vulgare* var. *sudanense* is V_1V_1, and that *S. halepense* originated as a segmental allopolyploid (STEBBINS 1947), containing the V_1 genome plus one designated as V_2, which was largely homologous with V_1, but differed from it with respect to a number of chromosome segments. The evolution of *S. halepense* after its origin would have involved heterogenetic association between V_1 and V_2 chromosomes, with natural selection for fertility tending to establish a balance which approached autopolyploidy in respect to factors affecting fertility, but which was still allopolyploid to the extent that morphological characters of the V_2 species were retained. The genomic constitution of modern *S. halepense* might therefore be designated as $V_{12}V_{12}V_{12}V_{12}$. This would agree with the external morphology, fertility, and nearly autopolyploid cytological behavior characteristic of *S. halepense*. The original hybrid between autotetraploid *S. vulgare* var. *sudanense* and *S. halepense* would have received two V_1 genomes from the former, and $V_{12}V_{12}$ from the latter, and so would have the constitution $V_1V_1V_{12}V_{12}$. This constitution would give it a slight degree of genetic unbalance, and so would explain the high degree of variability of its offspring in both external morphology and fertility. Most of its gametes with the reduced chromosome number would be non-functional, and polyhaploids would be expected only rarely, as was found to be the case. Furthermore, the V_1 and V_{12} chromosomes would have exchanged genes through crossing over during the four generations between the original F_1 hybrid and the progenitor of the present polyhaploid, so that one would expect the latter to have received mostly chromosomes of the V_1 type, but a few of them which contained blocks of genes derived from V_{12}. The polyhaploid, therefore, would be expected to appear and behave like a

strain of Sudan grass which had obtained genes by introgression from *S. halepense*. This is exactly the case, and therefore supports the assumptions which have been made concerning the genetic nature of *S. halepense*, and the events which took place prior to the formation of the polyhaploid.

SUMMARY

1. One culture of the F_6 generation from a hybrid between autotetraploid *Sorghum vulgare* var. *sudanense* (2n = 40) and *S. halepense* (2n = 40) consisted entirely of plants with 2n = 20 chromosomes, and presumably arose from a tetraploid F_5 plant through the parthenogenetic development of an egg with the reduced chromosome number.

2. In general morphology, the polyhaploid plants resembled tetraploid plants of the same generation, but were taller, had fewer tillers, broader leaves, more numerous panicle branches, and were more weakly perennial.

3. The chromosomes usually paired normally into 10 bivalents at meiosis, but occasional univalents were observed, leading to irregularity at later stages of meiosis, and somewhat reduced pollen fertility.

4. The morphology and chromosome behavior of the polyhaploids suggests that their F_5 parent was nearly autotetraploid, but contained some differences between its four genomes with respect to chromosome segments. This indicates that *Sorghum halepense* originated as a segmental allopolyploid containing two genomes derived from *S. vulgare* and two genomes derived from some related species with chromosomes partly homologous to those of *S. vulgare*.

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LITERATURE CITED

ELLIOTT, F. C., and C. P. WILSIE, 1948 A fertile polyhaploid in *Bromus inermis*. J. Hered. **39**: 377–380.

GARBER, E., 1944 A cytological study of the genus *Sorghum:* subsections *Para-Sorghum* and *Eu-Sorghum*. Am. Nat. **78**: 89–94.

GERSTEL, D. U., and WM. MISHANEC, 1950 On the inheritance of apomixis in *Parthenium argentatum*. Bot. Gaz. **112**: 96–106.

HÅKANSSON, A., 1924 Über die Chromosomenzahl einiger *Oenothera gigantea*-Pflanzen. Hereditas **5**: 93–96.

HUSKINS, C. L., and S. G. SMITH, 1934 A cytological study of the genus *Sorghum* Pers. II. The meiotic chromosomes. J. Genet. **28**: 387–395.

KATAYAMA, Y., 1935 Karyological comparisons of haploid plants from octoploid *Aegilotriticum* and diploid wheat. Jap. J. Bot. **7**: 349–380.

MYERS, W. M., 1947 Cytology and genetics of forage grasses. Bot. Rev. **13**: 319–421.

RANDOLPH, L. F., and H. E. FISCHER, 1929 The occurrence of parthenogenetic diploids in tetraploid maize. Proc. Nat. Acad. Sci. **25**: 161–164.

STEBBINS, G. L., JR., 1947*a* Types of polyploids: their classification and significance. Advances in Genetics I: 403–429.

STEBBINS, G. L., JR., and M. KODANI, 1944 Chromosomal variation in guayule and mariola. J. Hered. **35**: 163–172.

THE ACTION OF ALLELIC FORMS OF THE GENE *A* IN MAIZE. IV. ON THE COMPOUND NATURE OF A^b AND THE OCCURRENCE AND ACTION OF ITS A^d DERIVATIVES

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PRELIMINARY evidence on changes of the A^b factor in A^b/a heterozygotes (LAUGHNAN 1949) was interpreted as indicating that this allele is compound in the sense that it consists of at least two factors which, though they cooperate in the determination of the A^b effect, are separable by an infrequent crossover event. This argument was based on an observed high degree of association between the occurrence of changes of A^b, which has a purple effect, to A^d, an intermediate allele, and crossing over within an adjacent segment, 13 units in length and marked at its right-most extremity by the gene *et* (etched endosperm). In view of this coincidence the crossover event was argued to occur at the A^b " locus " between two components designated alpha (a) and beta (β). A^d was considered synonymous with alpha, the leftmost or proximal component, since the A^d-bearing strands carried predominantly the recombinant allele of *et*.

The present report deals with an analysis of the mode and frequency of occurrence, from A^b/a individuals, of larger numbers of A^d derivatives. Recent studies utilizing more efficient marker genes have localized the pertinent crossover event and indicate that some mechanism other than crossing over is responsible for the occurrence of a small proportion of the A^d derivatives.

CROSSING OVER AS A BASIS FOR THE ORIGIN OF A^d FROM A^b AND THE LOCALIZATION OF THE CROSSOVER EVENT

The original report (LAUGHNAN 1949) dealt with 41 independently occurring A^d cases derived from A^b/a individuals which were heterozygous for the marker genes lg_2 and *et*. Though only seven of the cases had received a thorough analysis at the time, it was apparent from endosperm classifications that 36 of the 41 A^d-bearing strands carried the recombinant allele of *et;* only 13 percent recombination is expected if there is no relation between crossover event and the change to A^d. The strand constitutions of these cases is given in the first four rows of table 1 together with additional cases of more recent origin. Of the 69 A^d-bearing strands tracing their origin to A^b/a individuals heterozygous in various combinations for *lg* and *et*, 62, or 90 percent, are crossovers in the A^d-*et* segment.

Since most of the A^b derivatives carry the *et* allele which was linked with *a* in the parent individuals the possibility may be considered that these changes originate from recessive *a* without crossing over at the *A* locus. Two types of

TABLE 1

Constitutions of 79 A^d-bearing strands from A^b/a individuals marked with lg and et or with et alone.

Egg-parent constitution	Number of A^d cases	Non-crossovers	Crossovers		
			Region 1 (lg-A^d)	Region 2 (A^d-et)	Regions 1 and 2
A^b *lg a et*	57	2	4	41	10
lg A^b *et/a*	2	0	0	2	0
A^b *et/lg a*	1	1	0	0	0
lg A^b*/a et*	9	0	0	3	6
Totals	69	3	4	46	16
A^b*/a et*	9	1		8	
A^b *et/a*	1	0		1	
Totals	10	1		9	

evidence are against this interpretation. The first of these, already reviewed (LAUGHNAN 1949), is based on distinctive effects of the A^d allele, a type which has never occurred as a mutant from a in spite of ample opportunity for its observation and isolation. The derivatives reported in the present study possess these effects, evidence for which is presented in another section. Direct evidence against the origin of these derivatives from a is obtained from consideration of the lg constitutions of the A^d-bearing strands given in table 1. Forty-nine of these strands (71 percent) carry the lg marker which was linked with A^b in the parent individuals. Since the lg-a recombination frequency is *ca.* 0.33 it is apparent that the A^d derivatives are not to be explained as mutations of a.

The interpretation of A^b which emerges from a consideration of the foregoing data is represented diagrammatically in figure 1. According to this hypo-

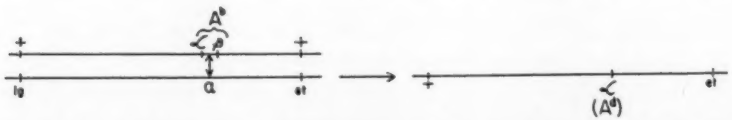

FIGURE 1.—Tentative scheme to account for the isolation of $A^d(a)$ from A^b/a individuals. The alpha component is separated from beta by a crossover between the component members of A^b.

thetical model which is adopted tentatively for the interpretation of other experiments reported here, the A^d derivatives arise by a crossover event which separates alpha from beta and results in a strand which carries only alpha (A^d).

Foregoing evidence on the lg and et constitutions of strands which indicates that A^d is the proximal component of A^b validates the use of A^d itself as a proximal marker in any experiments in which (1) the parent individuals are heterozygous for A^b and (2) the derivatives are identified on the basis of subsequent tests as valid cases of A^d. Thus in rows 5 and 6 of table 1 are recorded the constitutions of A^d-bearing strands from A^b/a individuals hetero-

zygous for *et* but not involving *lg* as a proximal marker. Nine of the ten strands carry the recombinant *et* allele from which it is inferred that A^d traces its origin in these cases to a crossover between the alpha and beta components.

Additional evidence in support of the scheme represented in figure 1 is obtained from experiments in which changes to A^d were obtained from A^b/a plants heterozygous for the translocation 2–3d and for *et*. The break-point of this interchange is proximal to *a* (ANDERSON and BRINK 1940), the *T-a* segment having a length of 7.1 crossover units as measured in the interchange heterozygote. Table 2 gives the constitutions of 11 A^d-carrying strands from this source; all of these have the proximal marker gene which was carried on the A^b chromosome of the parent and all but one are crossovers for the *T-et* segment.

Though it is apparent from the data presented in tables 1 and 2 that most of the occurrences of A^d are dependent on a crossover at some point within the *a-et* segment the data do not provide empirical evidence for the precise localization of this crossover event between component members of A^b as

TABLE 2

Constitution of 11 A^d-bearing strands from A^b/a individuals marked with T 2-3d and et.

Egg-parent constitution	Number of A^d cases	Non-crossovers	Crossovers		
			Region 1 (T-A^d)	Region 2 (A^d-*et*)	Regions 1 and 2
TA^b *et*/*a*	3	0	0	3	0
A^b *et*/*Ta*	8	1	0	7	0
Totals	11	1	0	10	0

represented in figure 1. It will be shown by formal argument that the pertinent crossover must in fact occur at the A^b "locus"; experimental evidence for its localization within a small segment which includes A^b will be presented.

On an alternative basis it may be argued that the crossover event in question takes place at the locus of a modifier somewhere between A^b and *et* and is such as to give rise to a state of that locus which modifies the purple expression of the A^b gene to the dilute phenotype characteristic of the A^d allele. Clearly such a scheme does not require a change in A^b nor that A^b be compound, and would account for the occurrence of modified expression as due to a crossover event. However, since such exceptional dilute individuals must be argued to carry a chromosome (contributed by the pollen parent) with the normal condition for the modifier it is expected that their selfed or suitably crossed progenies should, through recombination, produce some offspring which carry A^b, are without the active modifier and are therefore purple in phenotype. Their frequency of occurrence would be a function of the map distance between A^b and the hypothetical modifier locus. Since such purple individuals have not been observed among thousands of offspring from dilute plants this interpretation is difficult to defend unless it is argued that the modi-

TABLE 3

Summary of linkage data pertinent to the placement of the sh_2 factor with respect to the factors a and et.

Constitution of the heterozygous parent	Total crossovers in region 1	Constitutions of region 1 crossover strands			
		A^b sh Et	*A^b sh et*	*a Sh Et*	*a Sh et*
A^b et/a sh	22	7*	0	0	15*

*The discrepancy between numbers of crossover strands carrying A^b and *a* is an apparent one only. The shrunken character is associated with susceptibility to fungi attacking the kernels on the ripening ear. Since it was necessary to classify *et* on the basis of its virescent seedling effect, only those A^b *sh* crossover cases which survived germination are included here.

fier is closely linked with A^b. Under these conditions the modifier itself comes under suspicion as a component of A^b. In fact the beta component is argued as a closely linked factor with purple effect and may, if preferred, be viewed as the locus of a closely linked modifier. Pending further evidence on the related origin of alpha and beta from an evolutionary standpoint it makes little difference which view is adopted.

Direct evidence for the localization of the crossover event responsible for the occurrence of the A^d exceptions is available from experiments in which a recently discovered factor for shrunken endosperm designated sh_2 (MAINS 1949) was employed. Mains placed sh_2 in the third linkage group 0.25 units from *a* but it was not determined whether it lies on the *lg* or on the *et* side of *a*. The data of table 3, giving the constitutions of 22 region one crossover strands from individuals heterozygous for the factors *a, sh* and *et,* indicate that *sh* lies to the right of *a,* that is, between *a* and *et*. Table 4 summarizes information on the constitution of A^d-bearing strands from A^b/a individuals heterozygous for *sh*. Crossing over is clearly responsible for the origin of most of the A^d cases since all except four of the seventy-one strands are crossovers in region 2 (A^d-*sh*), whereas only one in eight hundred is expected to have this

TABLE 4

Constitution of 71 A^d-bearing strands from A^b/a individuals marked variously with lg, T, sh_2 and et.

Egg-parent constitution	Number of A^d cases	Non-crossovers	Crossovers			
			Region 1 (lg-A^d)	Region 2 (A^d-sh)	Region 3 (sh-et)	Regions 1 and 2 (lg-A^d-sh)
lg A^b et/a sh	39	3	0	26	1	9
A^b et/a sh	15	0	.... (T-A^d)	15	0	....
T A^b et/a sh	2	0	0	2	0	....
A^b/a sh	15	0	....	15	....	....
Totals	71		Region 2 crossovers: 67			

constitution if crossing over is not involved. Furthermore the crossover event is localized within a segment less than one unit in length which includes the A^d-β interval.

ON THE OCCURRENCE OF A^d WITHOUT CROSSING OVER

Among the 161 A^d cases treated in tables 1, 2 and 4, all but 13 were coincidental in occurrence with a recombination for the distal marker gene. While this indicates that crossing over is the basis for the origin of most of the A^d derivatives an explanation for the 13 cases (8 percent) which are apparent non-crossovers is required. It will be recalled that in earlier experiments in which the gene *et* was employed as a marker, 5 of the 41 A^d cases were non-recombinants for *et*. Because of the extent of the segment involved it was not possible to rule out double exchanges as a basis for these cases, one crossover occurring between the components of A^b, thus isolating A^d, another somewhere between this locus and *et* to reconstitute the parental combination.

However, experiments employing both *sh* and *et* as markers now make it clear that such double exchanges, if they occur at all, are so infrequent as to fail in explaining all but a small proportion of the non-crossover cases. The data in the first three rows of table 4 are pertinent to this argument. Here are listed, among others, 52 A^d cases which trace their origin to a crossover giving a recombination for *sh*. However, each of these strands is a non-recombinant for the *sh-et* segment. Likewise, the data in table 3 may be reconsidered for their bearing on this question; in all cases the strands represent crossovers between A^b and *sh*, which, because the segment involved is short, may be held to simulate a crossover between the A^b components. Again all of the strands, 22 in number, are non-crossovers for the *sh-et* segment, from which it appears, taken with the 52 similar cases reported above, that a crossover event in the neighborhood of the *A* locus reduces the chance of a coincidental crossover between *sh* and *et* and involving the same strand, and may even eliminate that possibility. It appears highly unlikely that the five A^d cases which are apparent non-crossovers may be accounted for on the basis of double exchange.

Whether or not a small proportion of the apparent non-crossover A^d cases from an *et*-marked background arise as a result of double crossovers within the marked segment, there is direct evidence for their occurrence without crossing over between the component loci of A^b. Four cases of this sort (see row 1, table 4) from egg parents having the constitution *lg* A^b *et*/*a sh* have been analyzed. As indicated in the table, three of the emergent strands had the constitution *lg* A^b *et* (non-recombinant) while the fourth was a recombinant for *et* only (*lg* A^d). It is apparent that legitimate crossing over cannot be the basis for the isolation of A^d in these instances since the constitutions of these strands would, on that argument, call for the occurrence of a double exchange within a segment (A^d-*sh*) less than one crossover unit in length.

Additional evidence in support of a second mechanism for the origin of A^d derivatives is available from experiments employing hemizygous A^b plants in which the deficiency *a*-x1 (STADLER and ROMAN 1948) is substituted for re-

TABLE 5

Frequencies of occurrence of A^d among gametes from plants carrying A^b but of otherwise heterogeneous constitutions.

Source	A^d Gametes tested	All A^d cases		Crossover A^d cases		Non-crossover A^d cases	
		Totals	Frequency ($\times 10^{-4}$)	Totals	Frequency ($\times 10^{-4}$)	Totals	Frequency ($\times 10^{-4}$)
Marked without translocation:							
A^b/a	45,141	30	6.65 ± 1.21	26	5.76 ± 1.13	4	0.89 ± 0.44
A^b/a	17,194	17	9.89 ± 2.39	16	9.31 ± 2.33	1	0.58 ± 0.58
A^b/a	30,780	27	8.77 ± 1.68	23	7.47 ± 1.55	4	1.30 ± 0.65
A^b/a	47,142	25	5.30 ± 1.06	25	5.30 ± 1.06	0	
A^b/a	19,730	15	7.60 ± 1.96	15	7.60 ± 1.96	0	
A^b/a	6,558	7	10.67 ± 4.03	6	9.15 ± 3.73	1	1.52 ± 1.52
A^b/a	7,288	6	8.23 ± 3.36	6	8.23 ± 3.36	0	
Totals	173,833	127	7.31 ± 0.65	117	6.73 ± 0.62	10	0.58 ± 0.18
Translocation marked:							
A^b/Ta	6,680	8	11.98 ± 4.23	7	10.48 ± 3.95	1	1.50 ± 1.50
TA^b/a	33,509	5	1.49 ± 0.67	5	1.49 ± 0.67	0	
Deficiency background:							
A^b/a-x1	36,958	2	0.54 ± 0.38	0		2	0.54 ± 0.38
Dp A^b/a-x/a-x	13,884	0		0		0	

cessive *a*. In view of the proven deficient nature of *a*-x1 (experiments in this laboratory indicate that the deficient segment of *a*-x1 includes the locus of sh_2) occurrences of A^d owing to crossing over between the A^b components are not expected in these experiments, or conversely, any A^d alleles which appear must arise by some mechanism other than crossing over. Among *ca.* 37,000 tested A^b gametes from *lg* A^b *et*/*a*-x1 individuals two were shown by subsequent analysis to have the constitution A^d *et;* one of these was a recombinant for the *lg* marker. In both cases tests have confirmed that the parent individuals did in fact carry the deficiency. Unfortunately, progeny tests of one of the derivatives are not available. For the other, however, subsequent analyses have ruled out illegitimate bases for its occurrence. Whatever may be the origin of these cases it is clear that they cannot arise by a legitimate crossover involving strands of homologous chromosomes. A discussion of possible mechanisms to account for them is included in another section.

FREQUENCIES OF OCCURRENCE OF A^d AS A FUNCTION OF GENETIC CONSTITUTION

The data presented here dealing exclusively with frequencies of occurrence of the A^d derivative furnish additional evidence, though indirect, in support of the compound nature of A^b. Further, the results of certain experiments employing the translocation as a genetic marker appear to be significant for the ultimate problem of homology of components and synaptic pattern.

Table 5 summarizes data on the frequency of occurrence of A^d derivatives of the two types (crossover and non-crossover in origin) among the gametes of A^b-carrying individuals from each of three major sources which are characterized by markedly different constitutions. The first of these includes plants all of which are A^b/a in constitution and which, though they were marked in various ways, did not carry the translocation. Each of the seven sub-groups represents either a single family or closely related families whose parents on each side bore a sib relationship (except sub-group 7 which is heterogeneous in makeup). Though all seven sub-groups have some distant ancestry in common the individuals in sub-groups 3, 4 and 5 have one parent not represented in the pedigrees of the other sub-groups. In spite of this heterogeneous background the frequencies of A^d crossover cases among these sub-groups are remarkably constant; of the 21 individual comparisons which are possible, none of the frequency differences is significant. There is no evidence from these data for a significant effect of modifiers on the frequency of crossing over within the alpha-beta segment.

The number of non-crossover cases of A^d (table 5, last column) is too low to permit a reliable analysis of sub-group differences. However, these cases are rarer in occurrence (10: *ca.* 174,000) by a factor of 12 than the A^d derivatives which are isolated by crossing over (117: *ca.* 174,000).

The combined data of the seven sub-groups of table 5 indicate an overall rate of occurrence for A^d from A^b/a individuals of approximately 1 per 1400 A^b gametes (127 cases). In contrast with this is the frequency of gametes

carrying A^d from plants heterozygous for the deficiency (table 5, row 11). Among *ca.* 37,000 tested gametes only two (one of these not thoroughly analyzed) carried A^d and both arose by some mechanism other than crossing over. This reduction in hemizygous plants is further evidence of the crossover basis for the origin of the majority of A^d cases from A^b/a individuals since there is no opportunity for homologous pairing of A^b in the hemizygote. Similar evidence is available from experiments using plants in which A^b is carried in a small duplication (STADLER and ROMAN 1948) and both rod chromosomes are deficient for the *A* locus (table 5, last row). In this case the A^b segment not only has no homologous region with which to pair but in addition the duplication competes at a disadvantage for synapsis with a rod chromosome. There was no A^d cases from individuals of this constitution among *ca.* 14,000 tested gametes.

As noted elsewhere, the translocation 2–3d has been employed in heterozygous condition as a marker in certain of the experiments reported here. Since the point of interchange of this translocation is seven crossover units proximal to *a* it was expected that its use would represent an improvement over *lg* which is too distant from *A* (33 units) to be of value in many experiments. However, this advantage as a more efficient marker is, under certain conditions, almost cancelled by its tendency to reduce the frequency of occurrence of A^d. Thus only 5 A^d cases (table 5, row 10), all of them crossovers, occurred among 33,509 tested A^b gametes from translocation heterozygotes which carried A^b on the interchanged third chromosome and *a* on the normal homologue ($T\,A^b/a$). This is a rate of 1:6700 to be compared with the frequency of 1:1500 (117 cases) for crossing over between α and β in A^b/a plants without the translocation; the difference in frequencies is highly significant ($P < 0.0001$). Thus the presence of the interchange linked with A^b produces a four- to five-fold reduction in crossover frequency within the α-β segment. Ordinarily this would be attributed to the generally observed effect of a heterozygous translocation in reducing crossing over in segments neighboring on the point of interchange. If this is the case a corresponding suppression of crossing over would be expected in A^b/a individuals carrying *a* in the interchanged chromosome and A^b on the normal homologue. However, the data from $A^b/T\,a$ individuals (table 5, row 9) indicate a crossover frequency higher than that from each of the seven sub-groups of A^b/a individuals not carrying the translocation. The evidence is against a suppressing effect of the interchange in this linkage phase; rather, it suggests that an effect, if any, operates to increase the crossover frequency, though the difference in rates is not significant. On the other hand crossing over between α and β is significantly higher ($P = 0.02$) in $A^b/T\,a$ plants than in $T\,A^b/a$ individuals (compare rows 9 and 10, table 5), indicating that the linkage phase of the interchange is a significant factor in modifying the frequency of exchange in the α-β segment. It follows that the suppression of this event in $T\,A^b/a$ individuals may not be attributed exclusively or even in large part to the mere presence of the heterozygous interchange.

Since the $T\,A^b/a$ and $A^b/T\,a$ plants in these experiments were not sibs the

possibility of a differential effect of modifier genes affecting crossing over must be considered. On this argument the lowered frequency of crossing over in $T\,A^b/a$ as compared with non-interchange A^b/a plants would be attributed to modifier genes from the translocation stock which reduce crossing over in plants of the former genotype. Because two backcross generations from crosses to non-interchanged stocks intervene between the cross which first introduced the translocation and the tested $T\,A^b/a$ individuals these latter may be considered to trace only one-eighth of their germ plasm to the translocation stock. Yet the $A^b/T\,a$ individuals, each of which carried an entire complement of chromosomes derived from the translocation source, have a normal frequency for the crossover event; these results are incompatible with the assumption of modifier genes to account for a lowered frequency of the crossover event in plants carrying the interchange.

Since it appears that neither the assumption of a general effect of the translocation nor a changed background of modifier genes is adequate to explain the lowered frequency of crossing over between α and β in the $T\,A^b/a$ plants as compared with $A^b/T\,a$ and non-interchanged individuals, data on crossing over in other segments neighboring on the translocation should be significant in indicating whether the effect is localized or is general for all regions near the translocation. In this connection preliminary studies suggest that the linkage phase of the interchange has no significant effect on the frequency of crossing over in the *T-a* segment. Similar analyses are being undertaken for regions distal to *A* but in view of the negative results for the *T-a* segment, in spite of its proximity to the point of interchange, it may be concluded tentatively that the effect is a localized one and may have special significance for interpretations of the complex nature of A^b.

STUDIES ON THE ACTION OF THE A^d DERIVATIVES

The A^d derivatives from A^b have been discussed in previous sections without considering their possible heterogeneity. Fortunately there are several distinctive criteria by which A^d may be distinguished from other known intermediate alleles of *A* (LAUGHNAN 1948). Since these tests require classification among first or second generation progenies of the tested individuals the analyses reported here are of cases occurring in the earlier experiments only. However, since there was no special selection of cases to be analyzed these may be assumed to be a random sample. The criteria for classification together with results of analyses are given below:

(1) The A^d allele, like A^b from which it is derived, produces a brown pericarp in the presence of *P*, the basic pericarp-color factor. Though all intermediate alleles at this locus have a similar effect, the brown pericarp of A^b and its derivatives is dominant to *A* which is associated with red pigmentation. Forty-one independently occurring A^d cases have been tested in compounds with *A*. Thirty-six of these originated in connection with the crossover event, the remaining five by some other mechanism. In each case heterozygotes with *A* exhibit brown pericarp indicating dominance of the A^d effect.

(2) A^d is competitive in compounds with other alleles having a higher level

of action; it detracts from, rather than adds to, the effect of these alleles. Thirty-nine of the A^d derivatives reported herein (including six non-crossovers) were tested in compounds with A^{br}. This allele is particularly suited to the present purpose since in A^{br}/a or A^{br}/Df individuals it produces a purple phenotype which is modified to maroon in the A^{br}/A^d heterozygote. All tested cases of the latter exhibited the competitive effect in families in which sib A^{br}/a plants were available for comparison.

(3) Although A^d is associated with a decidedly intermediate phenotype in plant and aleurone, there is no cumulative effect with increasing doses. Analyses have been conducted among the selfed progenies of hemizygous plants in which the effects of two doses in the vegetative tissues and three in the endosperm may be studied without the simultaneous dosage of a second allele (see MOHR 1919). The original seven A^d derivatives (one of them a non-crossover) have been tested in this manner; none shows a phenotypic response to increasing doses. In view of evidence (LAUGHNAN 1948) indicating that recessive *a* neither shows a cumulative effect with increasing doses nor influences the expression of intermediate alleles in heterozygotes, the progenies of selfed A^d/a individuals may be considered valid tests of a dosage effect of A^d. Such progenies are available as a result of routine analysis of the A^d cases reported here and none exhibits a dosage effect.

In view of the identity of the A^d derivatives with regard to these criteria it is not surprising to find that they are similar also in the level of effect which they produce. Twenty-one of the derivatives, including three non-crossovers, were compared in sib progenies with one of several of the original A^d cases; these studies indicate that the derivatives either are similar in their level of effect or vary so slightly that differences are undetectable.

THE OCCURRENCE OF STABLE AND MUTABLE A^d ALLELES

Except where hemizygous plants were employed all of the A^b-carrying individuals in the present experiment were heterozygous for recessive *a*. This gene, ordinarily stable, reverts frequently to alleles of higher level in the presence of the *Dt* factor (RHOADES 1938, 1941). Although other known *A* alleles are stable under this condition there is some argument for expecting the A^d derivatives which derive by crossing over to respond to *Dt;* thus if *a* is normally paired at meiosis with the beta component of A^b, a crossover between α and β is expected to yield a strand in which *a* is carried adjacent to, and to the right of, $\alpha(A^d)$. Barring a position effect this A^d, by reason of its adjacent *a*, should be mutable provided *Dt* is present. On the other hand only stable A^d alleles are expected if *a* pairs consistently with the alpha component of A^b. If synaptic preference is accepted as evidence of structural homology an analysis of the stable versus mutable nature of the A^d derivatives in the presence of *Dt* should give some indication of structural correspondence between *a* and the components of A^b and possibly between these components themselves.

At this time 53 of the A^d derivatives, including 5 non-crossover cases, have been tested for mutability. Three of these, all crossovers, exhibit the high rate

of reversion typical of *a* except that in these cases, as expected, the reversion areas occur on a pale rather than colorless background. In each case the mutable nature of A^d (hereafter designated A^d-m) is confirmed by the presence of reversions in endosperms of homozygous individuals.

In view of the low frequency of occurrence of the mutable condition the stable character of the five tested cases of A^d which were non-crossovers does not constitute critical evidence that the combination of a with a as a result of a special crossover event is the basis for mutability. However, other evidence supports this interpretation: (1) The mutability of A^d-m is not autonomous but, as with *a*, is conditioned by *Dt*. (2) Among gametes from homozygous A^d-m egg parents two mutants have been isolated. These are indistinguishable from *a;* they are associated with colorless aleurones and are mutable with *Dt*. This indicates that the A^d-m allele is mutable by reason of its aa constitution, an interpretation which receives additional support from the fact that the mutations of A^d-s (stable) to *a* which have been observed have in all cases been to forms which are stable with *Dt*.

DISCUSSION

Classical genetic investigations have identified localized *differences* throughout the length of homologous chromosomes. Since the existence of a gene is first established only through the finding of such localized modifications it is to these differences rather than to physically isolated units of function that the term gene-forms or alleles is legitimately applied. It is open to question therefore whether these differences reside in discrete entities as on the corpuscular theory of the gene or in relatively extended physical units within which crossing over is possible. Considering the latter possibility GOLDSCHMIDT (1950) has interpreted the lozenge "pseudo-alleles" (GREEN and GREEN 1949) as "aberrations" at different points within a relatively extended chromosome segment which is sensitive throughout to changes of the lozenge type. He considers that a non-localized genetic unit is required by the widespread evidence in *Drosophila* of similar mutant phenotypes associated with translocations, deficiencies and inversions occurring within regions corresponding to a number of salivary bands. However, these observations may be explained as readily on the assumption of discrete units if it is considered that the latter are not functionally isolated but require for the expression of wild type a specific pattern of interaction between themselves or their immediate products. In the absence of convincing evidence against it the hypothesis of the discrete determinant remains a useful model for the interpretation of experiments of the type reported here. In the discussions which follow the basis for assigning individuality to a component shall be its separability from another by crossing over though it makes little difference for the present considerations whether what corresponds to the component in a physical sense is construed as a localized entity or as a modification of a relatively extended determinant.

On the basis of evidence presented in a foregoing section the A^d-m allele is thought to consist ot two discrete components. One of these, because of the

similarity of A^d-s and A^d-m in all respects other than mutability, is identical with α, the constituent of A^d-s; the mutable nature of A^d-m resides in the second component, a, whose presence alongside α in A^d-m is attributed to a special synaptic pattern preceding the crossover event in A^b/a individuals. Hypothetical bases for the occurrence of the A^d-s and A^d-m alleles following crossing over are represented in figure 2 in which α and β are side-by-side components each occupying a member of a repeat segment. There is no evidence that other genes lie within these segments but in view of the precedent which the case of Bar (STURTEVANT 1925; BRIDGES 1936; MULLER *et al.* 1936) establishes, segments rather than components only, are represented here. If a is paired with α in the A^b/a heterozygote (figure 2 A) a crossover between α and β isolates the leftmost component which, because it lacks a appears stable with *Dt*. When a is paired to the right of α, however (figure 2 B), one of the crossover derivatives carries both α and a and by reason of

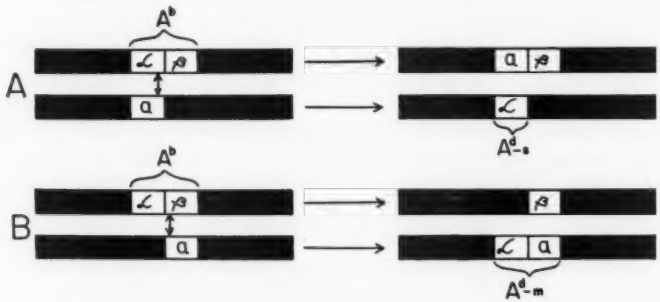

FIGURE 2.—Hypothetical schemes to account for the origin of (A) the A^d-s (stable) and (B) the A^d-m (mutable) derivatives following crossing over between non-sister strands in A^b/a individuals.

the latter, is mutable with *Dt*. It has already been noted that the A^d-m allele gives rise to a mutable form indistinguishable from a whereas the a mutants from A^d-s are stable with *Dt*. More rigorous proof of the αa constitution of A^d-m would call for the occurrence of mutable a from homozygous A^d-m by crossing over between α and a following oblique synapsis, an analysis which is underway at present.

If a may pair with either α or β it must be homologous with both of these components and it may be inferred that α and β are homologous with each other or lie in homologous segments. If a is represented by two components corresponding to α and β the argument remains unchanged since under these conditions it would still be necessary to assume alternative synapsis of a with α and β to account for the occurrence of stable and mutable A^d derivatives.

Since the argument that α and β originated through duplication is based on the foregoing interpretation of the origin of stable and mutable crossover derivatives, it is necessary to consider alternative hypotheses which might explain their occurrence without the assumption of a variable synaptic pattern.

Thus if it were argued that a is paired constantly between α and β a crossover between α and a would give rise to the A^d-m(αa) derivative while an exchange between a and β would produce the A^d-s(α) form. Though this scheme appears reasonable at first sight it involves the assumption of three homologous loci corresponding to α, a and β. Furthermore, because of the identity of A^d-m and A^d-s in all respects other than mutability, it requires that at least two of the hypothetical homologues be null forms insofar as A action is concerned.

Alternatively, if a pairs constantly with α, the stable and mutable A^d forms may be considered to arise as a result of " unequal crossing over " by which is meant here a lack of correspondence of the site of the break point in homologous strands involved in a crossover. Thus crossover breaks to the right of both α and a would result in A^d-s(α) while an unequal crossover, one break occurring to the right of α, the other to the left of a would give rise to A^d-m(αa). However, unequal crossing over in this sense, though once considered as a basis for mutation in general (SAX 1931), is not a likely event since it would require that crossing over be associated with some mutations of all genes. Moreover, evidence from studies of the Bar duplication, which have the advantage of critical cytological analysis, is opposed to this interpretation. The occurrences of normal and double-bar derivatives from B/B individuals (STURTEVANT 1925) are associated with crossing over in the Bar region. If these exchanges may be unequal in the sense implied above, cytological analysis would be expected to show discrepancies among the derivatives in the number of bands in the 16A region of the salivary chromosomes. The cytological studies of BRIDGES (1936) and of MULLER *et al.* (1936) indicate that the individual 16A segments of the normal and double-bar forms have the bands characteristic of that segment in the original Bar duplication. This in fact is the most convincing evidence that the changes of Bar trace to an " equal " (opposite) crossover following oblique synapsis.

Though it appears from the foregoing consideration that there are irregular synaptic patterns at the A locus in A^b/a individuals, this fact alone does not account for the discrepancy in frequencies of occurrence of the stable and mutable A^d forms; it will be remembered that only 3 out of the 54 tested cases were of the latter type. There are several possible explanations for this discrepancy though no evidence is available at present to distinguish between them. (1) It may be argued that while a is capable of synapsis with either α or β, an association with one of these is more frequent. If the degree of homology is the most important factor determining the synaptic pattern it might be expected that structural relationships between components would be revealed by an analysis of derivatives following crossing over. Thus if a tends to pair with α rather than with β, associations of the type illustrated in figure 2 A are expected more frequently than the α-β type of synapsis (figure 2 B). On this basis the preponderance of stable A^d derivatives would indicate a greater homology between a and α than between a and β. However, it would be dangerous to draw conclusions about relative homologies of components in

view of alternative explanations. (2) The synaptic patterns illustrated in figure 2 may occur with equal frequency but a, β and a may be considered to lie in the far-left region of their respective segments. This would account for the relatively low frequency of the mutable A^d form as compared with the stable type by assuming different lengths for the segments within which effective crossovers of the two types may occur, without requiring the assumption of a preferred synaptic association. (3) If it is assumed that a consists of two components homologous with a and β and that the mutable factor of a is located in the left segment, the rarity of A^d-m cases may again be explained; the mutable derivatives could be isolated only following oblique synapsis of one type while the A^d-s form would result from regular (opposite) pairing as well as from oblique synapsis of the other type when these are followed by a crossover between a and β.

Thus far β has been defined only as the component of A^b which is separated from a in the isolation of the A^d derivatives. From figure 2 it is evident that reciprocal crossover strands carrying β should occur among gametes of A^b/a individuals with a frequency equal to that of $a(A^d)$ isolations. However, no mutants other than those of the A^d type were observed in progenies which yielded 149 cases of A^d with a proven crossover origin. The exceptional A^d cases carrying the *et* marker which was linked with A^b in the tested parents occurred in frequencies far too low to consider them a reciprocal class of β isolations. Moreover, experiments in which appropriate markers proximal to A^b were employed indicate that these exceptional cases occur without an associated crossover. Since there is some reason to suspect that β is isoallelic with A^b the former may be undetectable in the present studies. This expectation is based on the following considerations: $A^b(a\beta)$ is associated with a purple plant phenotype; since $A^d(a)$ tends to reduce the level of wild type effect of purple acting alleles with which it is compounded, the effect of β would be predicted as at least the equivalent of A^b in producing purple phenotype (barring a position effect). In recognition of this possibility isolation of the beta derivative is now being attempted in experiments providing a special genetic background which will allow a distinction between A^b and β.

The interpretation of pairing relations given in figure 2 demands that most of the beta derivatives isolated following crossing over carry the a component at their left ($a\beta$). Since this derivative lacks the alpha component and carries a in its stead, its constitution may be verified by its failure to yield the A^d form and by the isolation of mutable a from the homozygote as a result of a crossover following oblique synapsis.

The beta component has further significance as a homologue possibly more closely related to a than is A^d. In the presence of *Dt* recessive a gives reversions which when isolated germinally are shown to have different levels of action (RHOADES 1941); those which are intermediate share a recessive brown pericarp effect (the brown pericarp of A^d is dominant to the red effect of A) and differ from A^d in regard to the additional criteria discussed previously. The reversions to wild type have a red pericarp effect in which they differ

from A^b (dominant brown action). Recessive *a* and its mutant forms thus fall in a different series qualitatively than that represented by A^d. Since the dominant pericarp effect of A^b is found in its isolated A^d component it may be argued that the beta component of A^b, rather than *a*, represents the *a* series. On these purely hypothetical grounds it might be expected that the beta component has greater pairing affinity for *A* and *a* than has A^d. However, from the observation that the great majority of the A^d derivatives from A^b/a plants are the stable type (do not carry *a*) it appears that *a* is most often paired to the left of β. In this connection experiments employing A^b/A plants have significance. Fifteen A^d derivatives have been obtained from this background and thirteen of these were associated in their occurrence with crossing over between A^d and *et*. Their frequency of occurrence is similar to that of A^d forms from A^b/a individuals. There is thus no evidence from these studies for preferred pairing between the beta component and either *a* or *A*. It should be emphasized, however, that the type of pairing, as deduced from the nature of derivatives isolated by crossing over, may not depend primarily on the relative homologies of components; other factors which might influence pairing relations have already been discussed and to these may be added the effect of gross chromosomal factors such as a translocation if, as seems likely from evidence now available, the linkage phase of the translocation 2–3d modifies the local pairing pattern in the A^b region.

If the occurrence of stable and mutable A^d alleles as a result of crossing over following different local pairing patterns is interpreted correctly to indicate the duplicated nature of A^b, its components may be considered to have a different arrangement than have those in the Star-asteroid repeat (LEWIS 1945) and those of the lozenge "alleles" (GREEN and GREEN 1948) in Drosophila. In these cases derivatives which would be expected if the member components may synapse obliquely were not isolated. This need not suggest that the components did not derive originally by duplication nor even that, having had such an origin, they have since evolved so that they no longer are capable of pairing. As various authors have suggested, if these duplications are reverse repeats (the segment corresponding to one component inverted with respect to the other) the strands resulting from crossing over within segments which are obliquely synapsed are expected to be dicentric and acentric alternatively and as such would not be included in functional eggs. The finding by LEWIS that Star, and probably asteroid, are included in the 21E1–2 doublet is in agreement with this interpretation and constitutes cytological evidence for the origin of these genes through duplication.

Since both stable and mutable A^d derivatives are viable and are carried on non-aberrant strands, the A^b duplication resembles Bar which is established cytologically as a direct tandem repeat. Further significance attaches to the Bar duplication since it represents an instance of the evolution of a gene with respect to an increase in gene number and a change in gene form, both occurring in recent time. Thus following the original occurrence of Bar (TICE 1914), now clearly established as a duplication, this "gene," in later studies

(STURTEVANT 1925), gave rise to B^i (infra-bar). Since infra-bar is cytologically indistinguishable from Bar it has been interpreted as a gene mutation, probably localized in one of the members of the Bar duplication. Thus B^i may be compared with the A^b duplication whose components must be argued as dissimilar. By further analogy with the Bar duplication and on the hypothesis of homology between α and β it is expected that homozygous A^b individuals should give rise to A^d following a crossover at the A^b " locus." Evidence from several independent sources suggesting that such a crossover derivative is obtained from A^b/A^b plants will be reported elsewhere. In addition certain other compounds involving A alleles are being investigated in an attempt to learn more about the structural correspondence (homology) between components, the possible existence of additional components of A^b, and the probable association of other known " mutational " steps with crossing over.

With regard to those A^d cases whose occurrence is not associated with exchange between homologous chromosomes in the A^b/a individuals no single explanation is favored by the evidence now available. An explanation of the occurrence of the non-crossover derivatives must account for their established identity with the A^d type which is isolated from A^b by crossing over. This requirement is met if it is assumed that they occur as a result of a particular type of gene mutation of the beta component. In view of the similarity in action of the non-crossover A^d cases both in comparison with each other and with the form which derives by crossing over, the postulated mutation of β is restricted to forms with null action. A critical test of this hypothesis is not feasible at this time since it is contingent on the recognition and isolation of the beta component following which it may be determined whether β mutates exclusively to a null allele and moreover whether it does so with a rate similar to that for the occurrence of the non-crossover A^d allele from A^b. Furthermore it must be assumed on this hypothesis that the postulated mutation of β is highly localized with respect to its time of occurrence. This is apparent from the fact that each of the 14 A^d cases of this type which have thus far been isolated occurred as a single exceptional individual in its respective family; clusters of A^d cases on individual ears have never been observed. Moreover, individuals sectored for purple and pale which would suggest post-meiotic mutation of β are either absent or are extremely infrequent in occurrence as judged from studies in which the genetic background provides a critical basis for their recognition. Thus the explanation which would account for the non-crossover A^d derivatives as due to mutation of the beta component imposes limiting conditions for the time, type and frequency of such a change.

Even in the event that all of these requirements are satisfied by independent studies on β there remains the problem of distinguishing between a gene mutation in this case and an aberration simulating gene mutation. Thus a change from A^b to A^d would be expected also following a physical loss of the beta component. If the present cases are interpreted as deficiencies the evidence indicates that the lost segment includes neither α on the left nor the closely linked normal allele of sh_2 on the right; in addition the homozygous sporo-

phyte is in all cases viable and gametophyte function is unimpaired. Under these conditions it is impossible to distinguish between an actual loss of β and a gene mutation of β to a null-acting allele.

As an alternative mechanism to account for the occurrence of the non-crossover A^d derivatives, sister-strand crossing over may be considered. Since this explanation calls for a separation of α from β which is in effect analogous to their separation by a legitimate crossover the observed identity of the A^d derivatives of crossover and non-crossover origins would be expected. On this hypothesis, however, since both of the sister chromatids involved have the constitution of $\alpha\beta$ it is apparent that $\alpha(A^d)$ could be isolated only as a result of unequal sister-strand exchange.

It is interesting to note that STURTEVANT (1925) has recorded several cases of changes at the Bar locus which were not associated with crossing over in that region. Crossing over between sister strands was considered a possible explanation for these changes but was not seriously contemplated since they could have been the result of contaminations. Moreover it was argued (STURTEVANT 1928) that if sister-strand crossing over occurred at all it "would be expected to give many more apparent non-crossover mutations than have been observed." However, in this particular case, and also in the case of A^b, the expectation for occurrences of sister and non-sister strand exchanges on a random basis may not be valid; since an unequal exchange is required it is not unreasonable to consider that, owing to their origin by reduplication, oblique juxtaposition of the Bar segments in sister strands may be relatively infrequent in comparison with such an orientation between non-sister chromatids. On this basis non-crossover "mutations" would be infrequent in occurrence as compared with those involving crossing over between non-sister strands.

There is no evidence for the occurrence of sister-strand crossing over from studies in Drosophila (MORGAN 1933; MULLER and WEINSTEIN 1933) employing structurally modified chromosomes which are expected to produce aneuploid gametes following sister-chromatid exchanges. Again it may be emphasized that these analyses do not permit the detection of sister-chromatid crossing over if the latter is relatively infrequent in occurrence.

Considering the lack of evidence in general for sister strand exchanges and the difficulties of testing the hypothesis objectively, this explanation to account for the non-crossover A^d derivatives may be favored only insofar as evidence suggests the elimination of alternative hypotheses.

The significance of the present studies for the problem of gene mutation may now be considered. Students of genetics and evolution regard mutations as the ultimate source of variation. Included among these are the so-called "gene mutations" by which are meant inherited changes at the molecular level within what is considered the gene. While there is at present no precedent for this kind of change the lack of evidence is not surprising considering that the present concept of the gene is based on transmission and action studies which, though they are interpreted in most quarters to indicate a linear arrangement of unit determinants, give no clue to the molecular configuration of the indi-

vidual unit and hence provide no basis for a definition of gene mutation in physical terms. Moreover extragenic alterations which give rise to inherited changes remain confused with gene mutations if there is no cytological or genetical basis for recognizing them as aberrations. In this connection it has been noted (STADLER 1946; STADLER and ROMAN 1948) that the nature and action of the gene as revealed in mutation studies may be inferred with least bias from investigations of spontaneous changes at specific loci. This view rests on an expected high frequency among induced mutants of extragenic changes simulating gene mutation.

If the evidence from the various studies of closely linked determinants with similar effects may be considered for its bearing on this particular problem it would be predicted that an assortment of spontaneous mutations at such a "locus" would include a share of extragenic alterations which, without the knowledge that a gene cluster is dealt with, may pass as gene mutations. Thus a rare crossover between duplicated loci having different but related effects may isolate a single component which would be taken as a gene mutation unless the region were efficiently marked. Even with such precautions a loss of one of the components would be scored as a gene mutation. Genes which change spontaneously to forms with intermediate effects or which mutate to alleles having a null effect with respect to one aspect of a phenotype but retaining their characteristic effect for another, have been suggested as favorable objects for mutation studies (STADLER 1941). This view is based on the consideration that such derivatives may not be confused with deficiencies. It now appears that this advantage does not hold for closely linked gene systems of the type reported here since a mutation to an intermediate form could occur as a result of a loss of one of the components which together determine a normal phenotype. Furthermore, even changes in such components which are valid gene mutations may lead to difficulties in interpretation. Thus if the components are not strictly identical in function they may be expected to give rise to an array of mutant forms which do not fit into a simple quantitative series or which are not linearly seriated with respect to several aspects of a phenotype or regions of its expression. In the absence of knowledge that a linked gene system is involved there is the danger of accounting for such divergent effects in terms of relatively more complex and obscure physiological reactions instead of at the gene level itself. The A^b gene is a case in point since it and its derivatives do not conform in action to most of the other known A alleles (LAUGHNAN 1948). It now appears that this divergent action may have as its basis the component nature of the "locus."

It has long been considered that point mutations include alterations other than gene mutations but indistinguishable from them. Though the accumulating evidence concerning closely linked genes with similar effects may add little in a positive way to the concept of gene mutation, it suggests additional phenomena which, with special analysis, may be removed from the class of gene mutations.

SUMMARY

1. From genetically marked A^b/a individuals 161 independently occurring changes to A^d were isolated. Of these 148 were recombinants for the distal marker gene. It is concluded that A^b consists of separable components here designated α and β. $A^d(\alpha)$ represents the left-most or proximal component of A^b.

2. Seventy-one of the A^d derivatives were isolated from A^b/a plants which were heterozygous for sh_2, a factor for shrunken endosperm located 0.25 units distal to *a*. Since 67 of these were recombinants for the *sh* factor, the crossover event which isolates A^d is localized within a segment less than one unit in length and including A^b.

3. Thirteen (8 percent) of the 161 A^d derivatives were carried on strands which were non-recombinant for the distal marker gene. Since four cases of this kind were obtained from plants heterozygous for sh_2, it is concluded that these are not the result of double exchanges between A^d and the distal marker. Moreover this explanation will not account for the occurrence of apparent non-crossover cases which were isolated from plants heterozygous for *et*, a more distant marker, since it is established that a crossover near the *a* locus greatly reduces, and probably eliminates, the chance of a second crossover in the *a-et* segment.

4. The occurrence of A^d in the absence of crossing over between α and β is confirmed by isolations of this allele from plants heterozygous for A^b and the deficiency *a*-x1.

5. Three alternative explanations are offered to account for the origin of these non-crossover A^d derivatives, namely unequal sister-strand exchange, mutation of the beta component or the physical loss of the latter. Though none of these is excluded by the evidence now available their occurrence as a result of mutation or loss of the beta component requires limiting conditions for the type and time of occurrence of this event.

6. The frequency of occurrence of A^d (1 per 1400 gametes) is relatively constant judging from studies in which seven sub-groups of A^b/a individuals were compared. However, the heterozygous condition of the translocation 2–3d (employed as a genetic marker in some experiments) produces a four- to five-fold reduction in crossover frequency within the a-β segment when A^b is carried on the interchanged chromosome ($T\,A^b/a$). No suppression of this event occurs in $A^b/T\,a$ plants. Since the linkage phase of the translocation does not influence the frequency of crossing over in the *T*-*a* segment this suppression is probably the result of a modification of the local pairing pattern in the A^b region related to the particular linkage phase of the interchange.

7. Extensive studies on the action of the A^d derivatives indicate that all tested alleles possess the characteristic dominant brown-pericarp effect, are competitive in compounds with A^{br} and fail to give a cumulative response with increasing doses; moreover they are similar in the level of type effect which they produce. With regard to these criteria there are no differences between

the crossover derivatives and those whose occurrences are not associated with crossing over.

8. Fifty-three A^d derivatives from A^b/a parents were tested for mutability conditioned by the *Dt* factor. Three of these, all crossovers, showed the high rate of reversion in the presence of *Dt* which is typical of recessive *a;* the remaining fifty cases proved to be stable. On the present interpretation the A^d-m (mutable) derivative has two components. One of these is alpha, identical with the proximal component of A^b. The other is recessive *a* which is responsible for the mutable character of A^d-m and whose presence alongside a is attributed to a special synaptic pattern preceding the crossover event in A^b/a individuals. This interpretation is supported by the reisolation of mutable *a* from A^d-m.

9. The occurrence of A^d alleles of both stable (a) and mutable (aa) types following crossing over in $A^b/a(a\beta/a)$ individuals is explained if it is assumed that there is a variable synaptic pattern at the locus; this leads to the inference that the components of A^b represent a duplication.

10. The significance which evidence on closely linked genes with similar effects has for the problem of mutation is discussed.

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LITERATURE CITED

ANDERSON, E. G., and R. A. BRINK, 1940 Translocations in maize involving chromosome 3. Genetics **25:** 299–309.

BRIDGES, C. B., 1936 The Bar "gene" a duplication. Science **83:** 210–211.

GOLDSCHMIDT, R. B., 1950 "Repeats" and the modern theory of the gene. Proc. Nat. Acad. Sci. **36:** 365–368.

GREEN, M. M., and K. C. GREEN, 1949 Crossing over between alleles at the lozenge locus in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **35:** 586–591.

LAUGHNAN, J. R., 1948 The action of allelic forms of the gene *A* in maize. I. Studies of variability, dosage and dominance relations. The divergent character of the series. Genetics **33:** 488–517.

1949 The action of allelic forms of the gene *A* in maize. II. The relation of crossing over to mutation of A^b. Proc. Nat. Acad. Sci. **35:** 167–178.

LEWIS, E. B., 1945 The relation of repeats to position effect in *Drosophila melanogaster*. Genetics **30:** 137–166.

MAINS, E. B., 1949 Heritable characters in maize. Linkage of a factor for shrunken endosperm with the a_1 factor for aleurone color. J. Hered. **40:** 21–24.

MOHR, O. L., 1919 Character changes caused by mutation of an entire region of a chromosome in *Drosophila*. Genetics **4:** 275–282.

MORGAN, L. V., 1933 A closed X chromosome in *Drosophila melanogaster*. Genetics **18:** 250–283.

MULLER, H. J., A. A. PROKOFJEVA-BELGOVSKAJA, and K. V. KOSSIKOV, 1936 Unequal crossing over in the bar mutant as a result of duplication of a minute chromosome section. C. R. (Doklady) Acad. Sci. U.R.S.S. 1 (X) 87–88.

MULLER, H. J., and A. WEINSTEIN, 1933 Evidence against the occurrence of crossing over between sister chromatids. Am. Nat. **67**: 64–65.

RHOADES, M. M., 1938 Effect of the *Dt* gene on the mutability of the a_1 allele in maize. Genetics **23**: 377–397.

1941 The genetic control of mutability in maize. Cold Spring Harbor Symp. Quant. Biol. **9**: 138–144.

SAX, K., 1931 Crossing over and mutation. Proc. Nat. Acad. Sci. **17**: 601–603.

STADLER, L. J., 1941 The comparison of ultraviolet and X-ray effects on mutation. Cold Spring Harbor Symp. Quant. Biol. **9**: 168–177.

1946 Spontaneous mutation at the *R* locus in maize. I. The aleurone-color and plant-color effects. Genetics **31**: 377–394.

STADLER, L. J., and H. ROMAN, 1948 The effect of X-rays upon mutation of the gene *A* in maize. Genetics **33**: 273–303.

STURTEVANT, A. H., 1925 The effects of unequal crossing over at the Bar locus in *Drosophila*. Genetics **10**: 117–147.

1928 A further study of the so-called mutation at the Bar locus in *Drosophila*. Genetics **13**: 401–409.

TICE, S. C., 1914 A new sex-linked character in *Drosophila*. Biol. Bull. **26**: 221–230.

AN IMMUNOGENETIC STUDY OF SPECIES-SPECIFIC ANTIGENS IN THE SERUM OF SPECIES OF STREPTOPELIA[1]

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EVIDENCE has been presented in previous communications from this laboratory (CUMLEY, IRWIN and COLE 1941; CUMLEY and IRWIN 1942) that the antigens in the serum of a species of doves (Pearlneck or *Streptopelia chinensis*), specific for that species as contrasted with either of two other species (Senegal, *St. senegalensis*, or Ring dove, *St. risoria*), segregated in backcross individuals in accordance with genetic expectation. Subsequent data have supported this finding and have indicated that the species-specific antigens in the sera of other pairs of species of birds from which species hybrids have been obtained, Senegal × Ring dove, and pigeon (*Columba livia*) × Ring dove, likewise are subject to genic influence (CUMLEY, IRWIN and COLE 1943; IRWIN and CUMLEY 1942). The purpose of the present paper is three-fold: (1) to demonstrate the individuality of the species-specific antigens of Pearlneck, as contrasted with Senegal, which have segregated in offspring produced by mating to Senegal the species hybrid between Pearlneck and Senegal, and certain backcross hybrids of this cross; (2) to demonstrate the individuality of the species-specific antigens of Pearlneck, as contrasted with Ring dove, which have segregated in a similar manner; and (3) to show what relation, if any, exists between the antigens specific for Pearlneck as contrasted with Ring dove and Senegal, respectively.

MATERIALS AND METHODS

Except for some minor variations, the immunological technics employed in this study were essentially the same as those described in detail in earlier investigations (CUMLEY and IRWIN 1942; and CUMLEY, IRWIN and COLE 1943). Rabbits were inoculated intravenously with the serum of Pearlneck, and the antisera thereby produced were tested by the "ring" precipitation test with the sera of Pearlneck, Senegal, and Ring dove. The highest dilution of antigens at which a visible reaction occurred was determined, the presence of a cloudy plane of precipitate at the antigen-antiserum interface within two hours being indicative of a positive reaction. Antibodies were absorbed by

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mixing the Pearlneck antiserum with the serum of Ring dove or Senegal. Multiple absorptions involving the serum of either Senegal or Ring dove, or both, plus that of one or more of the backcross birds, from the cross between Pearlneck and Senegal, or Pearlneck and Ring dove, also were made.

Usually three such absorptions were sufficient to exhaust the antibodies, the first lasting 15–45 minutes at room temperature (25–28°C), the second ½ to 3 hours at room temperature and then 14–18 hours (overnight) in the refrigerator (2–6°C), and the third, if necessary, about 2 hours at room temperature. Previous reports have varied somewhat in the time and quantity of serum required for complete absorption. Actually, different antisera and antigen-antibody combinations required different treatments to assure optimal results. In the present study, 1.0 cc of the anti-Pearlneck serum was completely absorbed by 0.8 cc of Ring dove serum divided into two absorptions: the first included 0.5 cc of serum antigen and lasted 20 minutes at room temperature; after centrifugation, 0.3 cc of the Ring dove serum was added to the supernatant fluid and held 3 hours at room temperature and then overnight in the icebox. In the double absorptions, the requirements varied, depending upon the serum of the bird used, but in general 1 part of Ring dove serum and 1 part of the serum of a backcross bird were required to absorb 1–2 parts of the antiserum. After the antiserum was found to be completely absorbed, which was ascertained by testing the absorbed fluid with the antigen or antigens used in absorption, the "reagent" thus obtained was tested with the sera of selected hybrids and backcross hybrids. In these tests the serum antigens were tested in the dilutions 1:2 and 1:4, and in some instances at higher dilutions; only infrequently were the end-points of the reaction determined. However, since major emphasis was placed on the presence or absence of a reaction, the antigens (sera) were seldom diluted beyond 1:16; most of the sera that reacted to the absorbed reagents gave their clearest reactions at the antigen dilutions 1:2–1:16.

Of three anti-Pearlneck sera produced, only one (49F6) appeared to be of sufficient strength to justify thorough investigation, although some tests were made with another antiserum (46F3). In the first set of experiments the Pearlneck antiserum (49F6) was absorbed with the serum of Senegal, and of Senegal plus each of seven different backcross birds. These latter were the survivors of the group of 14 quarter Pearlneck–three quarters Senegal, and one eighth Pearlneck–seven eighths Senegal individuals whose sera were the object of a previous investigation (CUMLEY, IRWIN and COLE 1941). Absorptions and subsequent tests were carried out with the sera of the seven birds, with results as shown in table 1.

The second set of experiments involved the testing of the sera of backcross hybrids from the Pearlneck × Ring dove cross. The anti-Pearlneck serum was absorbed with the serum of Ring dove, and of Ring dove plus one or more of the different hybrid or backcross birds. The serum from each of 27 hybrids and backcross hybrids was tested with the various absorbed fluids produced in the course of these experiments. The reactions of the sera of only 21 of

these birds are shown in table 2; the sera of the remainder gave no reaction when tested against the reagent produced by absorbing the anti-Pearlneck serum with the serum of Ring dove. Only those families of birds in which specific Pearlneck precipitinogens were found to be segregating are shown in the table. Unfortunately most of the birds whose sera were studied previously (CUMLEY and IRWIN 1942) were dead at the time the present investigations were begun, but it was possible to obtain offspring of several that in the earlier tests had been shown to possess Pearlneck-specific serum antigens.

In the third set of experiments, the anti-Pearlneck serum was absorbed with the pooled serum of either Ring doves or Senegals, and then tested with the sera of individual backcross birds of the two species crosses—Pearlneck and Ring dove, and Pearlneck and Senegal. The antiserum to Pearlneck also was absorbed with the serum of Ring dove and of Senegal plus that of certain of the backcross birds from each of the two species crosses, in an effort to determine their mutual interrelationships. The precipitations observed during the course of this last set of experiments were usually much less pronounced than had been previously observed. The Pearlneck antiserum had been stored at refrigerator temperature for two months, and the antibodies to the species-specific components of the various sera used were reduced in reactivity, in fact, certain antibodies seemed to have disappeared. Much more attention was given to the possibility of overabsorption than was required in the previous tests, and only the reactions of those absorbed sera are reported for which this phenomenon could be ruled out.

It should be pointed out that the dependability of the reactions of the reagents, following absorption, was ascertained by the application of certain criteria outlined by KRUMWIEDE, COOPER and PROVOST (1925). For example, when two reagents were prepared by the absorption of an antiserum with the sera of two different birds whose serum antigens were judged to be equivalent (*i.e.*, each serum removed precipitins from the antiserum for itself and also for the other), all subsequent tests of these two reagents should have yielded equivalent results with other sera, either for the presence or absence of reaction. Any lack of agreement of the reactions of various sera with the reagents which were supposedly equivalent usually implied errors in technic.

EXPERIMENTAL RESULTS

Segregation of specific Pearlneck substances in the serum of backcross birds of the Pearlneck-Senegal cross

As in the previous experiments (CUMLEY, IRWIN and COLE 1941), it was found that the sera of both Pearlneck and Senegal reacted equally (antigen dilution of 1:8000) with unabsorbed Pearlneck antiserum. However, following complete absorption with Senegal serum, the "reagent" or "test-fluid" so produced would react strongly and consistently with the serum of Pearlneck at dilutions of this antigen greater than 1:32 (column 2, table 1), whereas no trace of a reaction was observed with the serum of Senegal. These results corroborate those previously obtained (*loc. cit.*) that there are in Pearlneck

antiserum at least two qualitatively distinct antibodies—at least one which is a counterpart of the serum antigens " shared " by Pearlneck and Senegal, and another which is " species-specific " for Pearlneck in contrast to Senegal.

Furthermore, as is shown in table 1 (column 2) the serum of each of five backcross hybrids (D820V2, E11U, D967Q, Y, and Y_2) was reactive with the reagent for specific Pearlneck substances, while that of each of two backcross hybrids (D967L2 and E132J2) was not. These results paralleled those of the previous report, except that in the former report the serum of D967L2 was strongly reactive with each of three anti-Pearlneck sera used in the tests. Whether this difference in the reactivity of this serum is due to (a) a mistake in recording the number of the bird, (b) individual differences in the response of the rabbit to immunization, or (c) sufficient overabsorption of the antiserum so that the antigen-antibody reaction in this instance was masked, is unknown.

The serum of each of these two birds with no reactivity, D967L2 and E132J2, was added to Senegal serum in separate absorptions of the Pearlneck antiserum, and the resulting reagents (columns 7 and 9) were then tested with the sera of the available backcross birds. No definite differences in reactivity of the serum of Senegal or the other backcross hybrids were noted with either reagent than with that prepared by absorption with Senegal serum only, except for a negative reaction at one test of the serum of D967Y, as compared with a definite reaction at another. It therefore appears that this Pearlneck antiserum (49F6) had no, or very faintly expressed, antibodies against specific Pearlneck substances in the serum of either of these two birds.

However, the reactions of the sera of the other five backcross hybrids with the reagents prepared by the use of the serum of each bird with that of Senegal in the absorptions revealed qualitative differences among these five birds in their content of specific Pearlneck substances. It may be noted in column 6 of table 1 that the serum of D967Y removed antibodies only for itself, in that the test fluid remaining after absorption by the serum of this bird plus that of Senegal reacted strongly with the respective sera of each of the other four hybrids. The constituent peculiar to Pearlneck in the serum of D967Y is called Ps-1 (Pearlneck serum abbreviated to Ps) as is listed in column 10 of table 1. The serum of D967Q also contains Ps-1, since this serum by absorption removed the antibody for D967Y (Ps-1) from the antiserum (column 5), and possesses another, Ps-2, by virtue of the precipitation observed following absorption of the antiserum with Ps-1 (column 6). And because the test fluid prepared by using the serum of D967Q (Ps-1.Ps-2) in absorption reacted strongly with the serum of D967Y2 (column 5), this serum of D967Y2 must contain a specific constituent in addition to Ps-1 and Ps-2, and this is called Ps-3. Further, since the reagent prepared by using the serum of D967Y2 in absorption did not precipitate (column 8) with the serum of either D967Q (Ps-1.Ps-2) or D967Y (Ps-1), the reciprocal requirements are satisfied that the serum of D967Y2 had a minimum of three specific Pearlneck components, namely, Ps-1.Ps-2.Ps-3. This reagent did react with the sera of both D820V2

TABLE 1

Precipitin reactions of the sera of backcross birds from the mating of Pearlneck and Senegal, using reagents prepared from an anti-Pearlneck serum.

Tests with serum of	Antiserum absorbed with the serum of Senegal or with serum of Senegal plus that of a backcross hybrid								Pearlneck specific precipitinogen proposed
	Senegal serum	D820V2	E11U	D967Q	D967Y	D967L2	D967Y2	E132J2	
Pearlneck	++	++	++	++	++	++	++	++	Ps-1.2.3.4.5
Senegal	0	0	0	0	0	0	0	0	None
				Birds of first backcross generation					
D820V2	++	0	++	+,0+	++	+	+	±	Ps-1.2.4
E11U	++	±	0	±	++	+	+	+	Ps-1.5
				Birds of second backcross generation					
D967Q	++	0	+	0	++	++	0	+	Ps-1.2
D967Y	++	0	0	0	0	+,0+	0	+	Ps-1
D967L2	0	0	0	0	0	0	0	0	None
D967Y2	++	++	++	++	++	++	0	++	Ps-1.2.3
E132J2	0	0	0	0	0	0	0	0	None
Column 1	2	3	4	5	6	7	8	9	10

Symbols: "++" indicates a strong reaction at an antigen dilution of 1:4 or higher; "+" indicates a definite reaction at an antigen dilution of 1:2 but faint or no reaction at a dilution of 1:4; "±" indicates an indistinct or faint reaction at 1:2 or 1:4; "0" indicates no reaction.

and E11U (column 8), showing that the sera of these two backcross birds contained one or more substances different from those (Ps-1.Ps-2.Ps-3) in D967Y2. As may be seen by the reactions of the sera of these two birds, D820V2 and E11U, in columns 3 and 4 of table 1, the antigenic constituents of their sera are dissimilar. The absorption with the serum of D820V2 removed the antibodies for both Ps-1 and Ps-2, since this reagent did not precipitate with the serum of either D967Q or Y, whereas the absorption with the serum of E11U (column 4) removed antibodies only for Ps-1 (D967Y, Ps-1), not for Ps-2 (D967Q, Ps-1.Ps-2). By the same kind of reasoning, the test fluid prepared by the absorption with the serum of D967Q (Ps-1.Ps-2) gave a definite reaction with the serum of D820V2—the lack of reaction at one test may be ascribed to overabsorption, or some other cause—and the serum of D820V2 may be assigned a new factor Ps-4, in addition to Ps-1 and Ps-2. Likewise, since the serum of E11U was precipitated in combination with each of the other test fluids, it contained at least one substance other than those previously proposed, which would be Ps-5 and the antigenic phenotype of E11U would be Ps-1.Ps-5.

Thus these immunological tests for qualitative differences in the sera of these five backcross hybrids indicate that there are at least four, probably five, different serum constituents peculiar to Pearlneck represented in the sera of these five birds. It is regrettable that progeny from these birds were not obtained so that further evidence of the separation of the specific antigenic constituents of the serum would have been provided. Lacking such a test, the qualitative differences in the sera of the birds in the D967 family are the best index of such separation. Thus, three of the four birds with Pearlneck specific constituents possessed Ps-1, two of the four carried the Ps-2 substance, and one of the four possessed the Ps-3 component. This kind of segregation, although inadequate to prove the independence of the substances, would be expected if each of the antigenic substances was determined by one or more genes on a single chromosome originally derived from Pearlneck. The independence of these specific antigenic substances of the serum from those of the blood cells has been noted (*loc. cit.*). For example, bird D967Y2 possessed Ps-1.Ps-2.Ps-3 in its serum, but had no antigenic characters in its red blood cells peculiar to Pearlneck.

The segregation of specific Pearlneck substances in the serum of backcross birds in the Pearlneck-Ring dove cross

When the Pearlneck antiserum (No. 49F6) was tested with the serum of Pearlneck and Ring dove, respectively, no serological differentiation between them was possible, as both sera precipitated at dilutions of 1:8000. These results paralleled those obtained in comparisons of the sera of Pearlneck and Senegal, as also did the reactions with Pearlneck serum of the test fluid prepared by absorption of the antiserum with Ring dove serum. This test fluid reacted (precipitated) not only with the serum of Pearlneck, as may be seen in column 2, table 2, but also with the serum of each of 13 of the 19 backcross hybrids (to Ring dove) from the first, second and fifth backcross generations.

TABLE 2

Precipitin reactions of the sera of backcross birds, from the mating of Pearlneck and Ring dove, with reagents prepared from an anti-Pearlneck serum.

Tests with serum of	Antiserum absorbed with the serum of Ring dove alone, or with the serum of Ring dove plus that of backcross hybrids											Precipitinogens proposed specific to Pearlneck	Specific Pearlneck cellular antigen	Row	1
	Ring dove serum	E698C	E743V	E743Y	E849A	E849D	E633G	E418K	E460L or E645H	E418K and E698C	E418K and E849D				
	Proposed precipitins remaining in the antiserum after absorption.														
	Pr-1 Pr-2 Pr-3 Pr-4	Pr-3 Pr-4	Pr-2 Pr-3 Pr-4	Pr-3 Pr-4	Pr-2 Pr-3 Pr-4	Pr-3 Pr-4	Pr-2 Pr-3 Pr-4	Pr-2 Pr-4	Pr-1 Pr-2 Pr-3 Pr-4	Pr-4	Pr-4				2
Pearlneck	++	++	++	++	++	++	++	++	++	++	++	Pr-1.2.3.4	+		3
Ring dove	0	0	0	0	0	0	0	0	0	0	0	None			4
	Hybrids of first backcross generation to Ring dove														
E698C	++	0	++	0	++	0	++	++	++	0	0	Pr-1.2	+		5
E743U	0		0		0		0		+	0	0	None	+		6
E743V	+	0	0	0	0	0	0	0	+	0	0	Pr-1	+		7
E743Y	++	0	++	0	++	0	++	++	++	f. tr.	f. tr.	Pr-1.2	+		8
E849A	++	0	0	f. tr.	0	0	0	0				Pr-1	+		9
E849D	++	0	++	0	++	0	++	++	++	0	0	Pr-1.2	+		10
	Hybrids of second backcross generation to Ring dove														
E633G	+	0	0	0	0	0	0	0	v. f.	0	0	Pr-1	None		11
E845A	+	0	0	0	0	0	0	0				Pr-1	d-7		12
E845B	0											None	d-7		13
E845D	0											None	d-7		14

TABLE 2 (*continued*)

Tests with serum of	Antiserum absorbed with the serum of Ring dove alone, or with the serum of Ring dove plus that of backcross hybrids											Precipitinogens proposed specific to Pearlneck	Specific Pearlneck cellular antigen	Row 1
	Ring dove serum	E698C	E743V	E743Y	E849A	E849D	E633G	E418K	E460L or E645H	E418K and E698C	E418K and E849D			
	Hybrids of fifth backcross generation to Ring dove													
E418K	++	++	+	+	+	+	++	0	±	0	0	Pr-1.3	d-4	15
E418M	±	0	0	0	0	0	0					Pr-1	None	16
E460L	0	0					0		0			None	d-11	17
E602D	±	0	0	0	0	0	0	0				Pr-1	d-4	18
E639A	0		0		0		0					None	None	19
E639D	0,±	0	0	0	0	0	0	0				Pr-1	None	20
E639G	+	0	0	0	0	0	0	0				Pr-1	None	21
E639H	±	0	0	0	0	0	0	0				Pr-1	None	22
E645H	0	0	0		0		0		0			None	d-11	23
Column 1	2	3	4	5	6	7	8	9	10	11	12	13	14	

Symbols: See table 1.

The species-hybrid parents of the hybrids of the first backcross generation were alive at the time of these tests, but not the parents of the hybrids of the second and fifth backcross generations which are listed in table 2. However, the sera of the respective backcross parents of these 19 backcross hybrids had previously been tested with other antisera (CUMLEY and IRWIN 1942), and had been found to be reactive with one or both of the test fluids previously used. Thus D458A2 was the hybrid parent, in the second backcross to Ring dove, of families E633 and E845. Two of the offspring of D458A2 (E633G and E845A) possessed a serum component specific to Pearlneck, by virtue of their reactivity with the specific test fluid, and two (E845B and D) did not. In the previous report (*loc. cit.*), the sera of six progeny of D458A2 were reactive with another specific test fluid, those of four others were not. Hence of the 14 progeny of the backcross hybrid D458A2, there were eight with reactive sera and six with non-reactive sera, suggesting strongly from genetic evidence alone the presence or absence of but one serum component specific to Pearlneck, and therefore a single causative gene or two or more linked genes.

The other backcross hybrids listed in table 2 belonged to families of which the following birds in the previous report (*loc. cit.*) were the parents: E40S was the hybrid parent of families E418 and E602, E211D of family E639, and E382E of families E460 and E645. Reactivity of the sera of the three progeny of E40S (E418K and M, E602D) was noted, of three of the four offspring of E211D (E639D, G and H), but not of either of the two progeny (E460L and E645H) of E382E. But whether the same or different specific serum components are possessed by the backcross birds in these families, as the presumed single constituent in birds E633G and E845A, is unknown without further immunological tests. Such tests require observations of the sera following absorptions of the Pearlneck antiserum with Ring dove serum plus that of a hybrid.

It may be noted in column 10, table 2, that the use of the serum of either of two backcross hybrids (E460L or E645H), with sera not reactive with the reagent for specific Pearlneck components (column 2), did not change significantly the reactions of the resulting reagent from the reactions obtained following absorption with the serum of Ring dove alone. However, when sera reactive with the reagent for specific Pearlneck components were used in absorption, differing specificities were revealed.

Thus the serum of the backcross bird E743V removed by absorption antibodies not only for itself, but this reagent did not react with previously reactive sera from the following birds (column 4): E849A, E633G, E845A, E418M, E620D, E639D, G and H. The sera of E698C, E743Y, E849D and E418K, however, were still strongly reactive with this reagent. Furthermore, parallel reactivities were obtained when reagents prepared by absorption with the sera of either E849A or E633G were used in the tests as those following absorption with the serum of E743V (see reactions in columns 4, 6 and 8, table 2). Absorptions were made, but are not listed in table 2, with the sera of the other six backcross birds of this group, and identical reactions to those

of columns 4, 6 and 8 were noted. From the results it appears that nine backcross birds listed in table 2 (E743V, E849A, E633G, E845A, E418M, E620D, E639D, G and H) possessed in their respective sera the same serum component, specific to Pearlneck in contrast to Ring dove. This component is called Pr-1, and is assigned to each of these nine hybrids, as listed in column 13, table 2.

Of the backcross birds given in table 2, there are 13 whose sera gave the reactivity expected if Pr-1 were in their sera, and six which did not. Two (E460L and E645H) of these latter six, however, cannot be included in these data, for it is not known whether the serum of their hybrid parent (E382E) contained Pr-1 or some other factor. This ratio of 13 offspring with Pr-1 to 4 without, from a backcross, is more suggestive of the segregation of two antigenic substances than of one, or that it was one component produced by either of two genes which are not linked. However, the significant deviation from the expected may not be surprising in such small numbers.

Although the serum of the backcross hybrid, E743V, did not remove by absorption the antibodies from the Pearlneck antiserum for the serum of hybrid E698C, it will be seen in column 3 of table 2 that the reagent prepared by using the serum of E698C was not reactive with the serum of E743V, containing substance Pr-1, nor with the sera of any of the other eight birds also possessing Pr-1. From such results, it appears that the serum of E698C possesses not only Pr-1 (*i.e.*, it removes the antibodies for Pr-1 by absorption) but at least one additional specific component, called Pr-2. The serum of E698C (Pr-1.Pr-2) removed antibodies not only for itself, but also for the serum of both E743Y and E849D. In fact, the three reagents prepared by the use in absorption of these three sera, respectively, produced parallel reactions in tests with the different sera (columns 3, 5 and 7), except that in one combination with the serum of E849A (Pr-1) a faint reaction was observed (column 5, row 9). Hence, from these reactions there appear to be a minimum of two components, Pr-1 and Pr-2, specific to Pearlneck in the serum of these three birds, E698C, E743Y and E849D, as given in column 13 of table 2.

It may be noted in table 2 that the serum of E418K (row 15) was reactive with each of the reagents prepared by removing antibodies to the specific components Pr-1 or Pr-1.Pr-2 (columns 3 to 8 inclusive). Hence the serum of E418K presumably contained at least one component other than those previously recognized, and this is called Pr-3. Since the serum of E418K by absorption removed the antibodies for the sera containing only Pr-1 but not for those possessing Pr-1.Pr-2 (see reactions in column 9), it must have the specific constituent Pr-1 as well as Pr-3.

The serum of E418K (Pr-1.Pr-3) was combined for absorption with that of either E698C or E849D (both having Pr-1.Pr-2), and the resulting reagents were reactive only with Pearlneck serum, and possibly with that of E743Y, as given in columns 11 and 12. If these faint reactions with the serum of E743Y may be disregarded there still remains to be explained the reactivity of the serum of Pearlneck with these two reagents. Since the antibodies for

TABLE 3

Tests of the relationships in backcross hybrids among antigens of the serum peculiar to Pearlneck in contrast to each of two species, Senegal and Ring dove.

Tests with serum of	Proposed specific Pearlneck components	Precipitin reactions with reagents prepared from anti-Pearlneck serum (No. 49F6) by absorbing with the serum of:					
		Ring dove	Senegal	Ring dove and Senegal	Ring dove, Senegal and E743Y	Ring dove, Senegal and D967Y	Ring dove, Senegal and D967Y2
Pearlneck		++	++	++	++	++	+
Ring dove		0	0	0	0	+	0
Senegal		+	0	0	0	0	0
		Backcross hybrids from the cross between Pearlneck and Ring dove					
E633G	Pr-1	0	0	0			
E698C	Pr-1.2	+	+	+	0	+	+
E743Y	Pr-1.2	+	+	+	0	+	±
E849D	Pr-1.2	+	+	+	0	+	+
E418K	Pr-1.3	0	0	0		0	0
		Backcross hybrids from the cross between Pearlneck and Senegal					
D820V2	Ps-1.2.4	++	+	+	±	±	±
D967Y	Ps-1	±	±	±	+	0	0
D967L2	None	++	0	0	0		0
D967Y2	Ps-1.2.3	++	±	±	+	+	0
E132J2	None	++	0	0	0	0	0
Column 1	2	3	4	5	6	7	8

Symbols: see table 1.

Pr-1, Pr-2 and Pr-3 presumably are not present in these test fluids, another antibody must be postulated in the reagent to account for this reaction of Pearlneck serum. The component of Pearlneck serum reactive with this antibody is called Pr-4, and it is realized that there may be more than one component involved. The serum of Pearlneck, therefore, as analyzed by this particular antiserum, possesses at least four components—Pr-1, Pr-2, Pr-3 and Pr-4—which distinguish it from the serum of Ring dove. Presumably each component is produced by the action of one or more genes on respective chromosomes originally derived from Pearlneck.

Relationships between antigens specific for Pearlneck as contrasted with Senegal, and those specific for Pearlneck as contrasted with Ring dove

Following the recognition of the different specificities in the backcross hybrids of the antigens of the serum peculiar to Pearlneck in contrast to Ring dove and Senegal, respectively, attempts were made to compare these different specificities of Pearlneck. Unfortunately, as described previously, at the time these comparisons were begun, at least some of the antibodies in this Pearlneck antiserum had decreased in potency. Those to Pr-1 and Pr-3 had largely or entirely disappeared, since the reagent for specific Pearlneck antigens of the serum prepared from it was no longer definitely reactive with the serum of one or more of the backcross birds presumably containing Pr-1 alone (as E633G), or with that of E418K carrying Pr-1.Pr-3, as may be seen in column 2, table 3.

It may be noted in table 3 (column 3) that the reagent prepared by absorbing this Pearlneck antiserum with the serum of Ring dove was precipitated by the serum of both Pearlneck and Senegal. Thus it is clear that Pearlneck shares with Senegal, to the exclusion of Ring dove, at least one serum antigen. While the absorption of this Pearlneck antiserum with Ring dove serum usually did not remove the antibodies for that of Senegal, absorption with Senegal serum sometimes, but not always, removed antibodies for the serum of Ring dove. These results suggest that the serum of Pearlneck and Ring dove contain very few, if any, antigenic specificities not also shared with Senegal. A somewhat similar finding concerning the antigens of the red blood cells has been reported (IRWIN and COLE 1940), in that Pearlneck and Senegal possess cellular substances to the exclusion of Ring dove, but Pearlneck and Ring dove have not been demonstrated to share more than a minimum of cellular antigens which are not also shared with Senegal.

There were four serum substances peculiar to Pearlneck, not in Ring dove, proposed to explain the data of table 2, and five proposed which differentiated Pearlneck from Senegal, according to the data of table 1. However, (a) if Pearlneck and Senegal share at least one antigenic substance of the serum which is not common to Ring dove, and (b) if Pearlneck and Ring dove do not have a common serum antigen to the exclusion of Senegal, Pearlneck serum must differ from that of Ring dove by at least one more serum com-

ponent than distinguishes Pearlneck from Senegal. The preceding evidence indicates that Pearlneck serum differs from that of Senegal by five components. Hence there should be six or more components by which Pearlneck serum differs from that of Ring dove. These are two more serum antigens than were identified in the backcross hybrids tested, but it is not unreasonable to believe that the backcross hybrids tested of this cross (Pearlneck × Ring dove) did not contain all the respective components of the serum of Pearlneck.

The precipitation of the reagent for Pearlneck specific substances of the serum, in contrast to Ring dove, as given in table 3 (column 3), with the sera of E698C, E743Y and E849D are presumably by virtue of the antibody against the Pr-2 substance. Furthermore, this Pr-2 substance is presumably not shared with Senegal, at least not completely, for the absorption with Senegal serum does not remove the antibodies for the sera of these three birds (column 4).

In all tests of the serum of E132J2, this serum has not been observed to contain any antigenic specificity peculiar to Pearlneck in contrast to Senegal. Therefore the reaction of the serum of E132J2 in combination with the reagent for Pearlneck specific antigens not in Ring dove (table 3, column 3) is evidence that this serum contains an antigenic component shared by and peculiar to both Pearlneck and Senegal in contrast to Ring dove. On the assumption that the antibodies to Pr-1 and Pr-3 of Pearlneck were not present in the Pearlneck antiserum at the time of these tests, and since, as explained above, Pr-2 is not shared by Pearlneck and Senegal, the antibody responsible in the reaction with the serum of E132J2 is specific either for Pr-4, or for an unrecognized serum constituent. The same process of reasoning could be applied in explanation of the differential reactivity of the serum of D967L2 (columns 3 and 4).

The use in absorption of the serum from both Ring dove and Senegal did not change appreciably the reactivities of the sera of the several backcross hybrids (column 5) from those obtained by the use in absorption of only Senegal serum (column 4). However, the reagents resulting from the absorption of Pearlneck antiserum with the sera of Ring dove and Senegal should permit the recognition only of the specificities of Pearlneck serum which differentiate it from both of these contrasted species.

The reagents prepared by further absorptions of Pearlneck antiserum by the serum of both Ring dove and Senegal in respective combination with that of E743Y (Pr-1.Pr-2), D967Y (Ps-1) and D967Y2 (Ps-1.Ps-2.Ps-3) did not by reactivities reveal identities among the sera in the Pr and Ps groups. The absorption with the serum of E743Y, presumably removing antibodies for Pr-1 and Pr-2, did not diminish the reactivity of the substance Ps-1 in the serum of D967Y, nor in the reciprocal test did the serum of D967Y (Ps-1) exhaust antibodies for the sera of E698C, E743Y, E849D (compare reactions in column 6 and 7). Thus it may be stated that Ps-1 appears to be a different substance than either Pr-1 or Pr-2.

Further, the absorption by the serum of D967Y2, presumably containing Ps-1, Ps-2 and Ps-3, did not exhaust the antibodies for those sera with

Pr-1.Pr-2 (see reactions in column 8). If this Pearlneck antiserum has lost its antibodies to Pr-1 so that the reactivity of reagents prepared from it with serum of backcross birds containing Pr-1.Pr-2 is by virtue primarily, if not entirely, of precipitation with the Pr-4 antigen, the removal of antibodies to Ps-1, Ps-2 and Ps-3 in the absorption with the serum of D967Y2 would leave antibodies to Ps-4 or Ps-5, or to both. These results therefore suggest that Pr-2 and either Ps-4 or Ps-5 are similar if not identical.

Obviously, unless each of these antigenic substances which represent specificities of Pearlneck serum in contrast to Ring dove and Senegal, respectively, are present singly in the serum of backcross birds, a precise comparison of their relationships is not possible. Unfortunately, further offspring from backcrosses to Senegal were not obtained, nor was this problem pursued further after the senior author joined the armed forces.

DISCUSSION

A segregation of antigenic components of the serum, peculiar to Pearlneck, has been observed following the production of species hybrids between Pearlneck and Ring dove, and Pearlneck and Senegal, and backcrossing these hybrids to Ring dove and Senegal, respectively. The differentiation by serological technics of the antigenic complexes in the sera of the two kinds of backcross hybrids lends support to the conclusion that one or more genes on each of four or five, possibly more, chromosomes of Pearlneck have effects on those antigenic constituents of the serum which serve to distinguish the serum of Pearlneck from that of either Ring dove or Senegal, or both. That these antigenic components of the serum are referable to the serum proteins is assumed in these studies, although direct evidence is not yet available on this point. However, with the information at hand, it is not possible to determine which of the serum constituents that distinguish Pearlneck from Ring dove are shared, in whole or in part, with Senegal, and *vice versa*. It appears reasonably certain, however, that such relationships exist.

As far as can be determined from the data, the genes acting on the specific serum components of Pearlneck are located on chromosomes independent of those carrying genes effecting the specific cellular antigens of Pearlneck. There are nine or ten chromosomes in Pearlneck with genes producing such antigens of the erythrocytes. If four or five chromosomes are the correct number with genes which are responsible for the species specificity of serum antigens, there are in all a minimum of 13 to 15 chromosomes of Pearlneck with genes having effects on the serum or cellular antigens specific to Pearlneck, in contrast to Ring dove. Thus almost half of the probable 34 ± 2 pairs of chromosomes of Pearlneck, assuming that it has the same number as the pigeon (*Columba livia*) and Ring dove (PAINTER and COLE 1943), carry genes making for species-specific effects.

On the assumption that both Pearlneck and Senegal have the same, or nearly the same, number of chromosomes, it appears that Senegal differs from Ring dove in approximately the same number of chromosomes with genic

effects specific to Senegal as does Pearlneck, nine or ten with genic effects on cellular antigens (IRWIN and CUMLEY 1947), and at least four with genic effects on cellular antigens (*loc. cit.*). In both Pearlneck and Senegal there are at least twice as many chromosomes with genic effects on the species specific antigens of the erythrocytes as there are chromosomes with genic effects on the specific antigens of the serum. This finding would agree with the proposal of BOYDEN (1943) that serum proteins are conservative hereditary traits. However, this difference in number of chromosomes with genic effects on the cellular and serum antigens, respectively, does not obtain in a comparison of Pearlneck and Senegal. The number of chromosomes of Pearlneck with genes making for specific effects which distinguish the serum of Pearlneck from that of Senegal is four or five, and there are four or five others with genes effecting specific substances of the blood cells (IRWIN 1949), probably eight to ten chromosomes in all with genic effects distinguishing Pearlneck from Senegal. One may assume that Senegal has the same number with genic effects differentiating it from Pearlneck. From present evidence then it appears that one species may differ from another by as many specific components of the serum as of the cells, and it does not seem unreasonable to believe that a species might differ from another by more specific components of the serum than of the blood cells.

There are certain differences in the technics of detecting the specific components of the serum and cells, respectively, which may invalidate to some extent any proposed parallelism of the number of chromosomes with genes having species-specific effects upon either serum or cells. For the detection of the cellular substances, the antiserum was diluted, usually 1:60, 1:120 or higher, and an antigenic substance which was not reactive at these or higher dilutions was not detected. Hence there may be specific antigenic substances detectable only at lower dilutions of an antiserum, and these may be as truly species-specific as those now recognized. For various reasons, it has not been an efficient laboratory procedure to attempt to detect such substances. In the search for specific components of the serum, however, it is the antigen itself (the serum) which is diluted, and the antiserum is held at a constant concentration, perhaps slightly diluted (1:2 or 1:4) or not at all beyond the diluting which unavoidably occurs during the process of precipitin absorption. One might hypothesize that slight changes in cellular antigens are therefore more likely to be detected than are those of the serum components.

If one compares the high proportion of successes obtained in engendering antibodies (agglutinins) by iso-immunization (the donor and recipient belonging to the same species) with red blood corpuscles, and what has been an almost universal failure of all attempts to date to detect antibodies (precipitins) following iso-immunization with whole blood or serum, it would be impossible to escape the conclusion that the evidence for genic effects on the blood cells is unmistakable, while by this criterion there is no conclusive evidence for the individuality of antigenic components of the serum. Such individuality of serum components should, of course, be expected within a species if single gene substitutions produce a demonstrable effect upon the antigenic

composition of the serum. The finding by the authors (1943) of individuality in the serum of humans requires the further demonstration of the heritability of the components responsible for the observed differences of the serum between individuals—the inability to continue the project to that end is another casualty of World War II. (It should be stated that, in attempts to duplicate the results from this laboratory, GOLDBERG and DE GARA (1948) failed to observe individuality in human serum. Further attempts to duplicate the previous results are definitely indicated.) On the other hand, the presence of normally occurring isoantibodies reactive with a cellular antigen in other individuals of the species, in contrast to its absence in the sera of other individuals, usually if not always in those carrying the cellular antigen, may be construed in all known cases (humans, cattle, and sheep) as an effect of a single gene on the serum proteins. The correlation of a different form of hemoglobin from the normal with the gene for sickle cell anemia, in heterozygous or homozygous form (PAULING, ITANO, SINGER, and WELLS 1949) is evidence for a genic effect upon a protein of the blood cells, but not on proteins of the serum.

Should detectable genic effects on the antigenic components of the serum be limited to specificities in the differentiation of species, and these be genetic in nature, the question immediately arises as to the type of genetic change involved. Unless small variations in serum antigens can be shown to accompany gene substitutions, and the evidence for this is hardly even suggestive as yet, one may then in explanation of species specificities of the serum be required to invoke the assumption of (a) a special kind of mutation or (b) that the change takes place only after the accumulation of numerous small mutations. This general subject has been discussed by LANDSTEINER (1945) and by the present authors (1942).

SUMMARY

Qualitative differences in antigens of the serum, presumably serum proteins, specific to Pearlneck were observed in the sera of backcross birds resulting from matings to Senegal of species- and backcross hybrids from the cross of Pearlneck × Senegal, and also from matings to Ring dove of species- and backcross hybrids from the cross of Pearlneck and Ring dove. The antigens of the serum appeared to be independent of those of the blood cells, indicating that the genes for one kind (as the serum antigens) did not influence the action of the genes for the other (the cellular antigens).

At present, the detectable genic effects on the antigenic components of serum seem to be limited to those specificities of the serum by which one species is differentiated from another. The few studies that have been made of the segregation in backcross offspring following hybridization between two species have yielded results which are understandable if interpreted as being gene determined, otherwise they do not fit any known pattern of explanation. Unless in the future small variations in antigens of the serum (proteins) are shown to accompany gene substitutions, it appears that the differences in specificities between closely related species may be due to (a) a special or macromutation, or (b) the accumulation of numerous small mutations.

LITERATURE CITED

BOYDEN, A. A., 1943 Serology and animal systematics. Amer. Nat. **77**: 234–255.

CUMLEY, R. W., and M. R. IRWIN, 1942 Immunogentic studies of species: segregation of serum components in backcross individuals. Genetics **27**: 177–192.

1943 Individual specificity of human serum. J. Immunol. **46**: 63–70.

CUMLEY, R. W., M. R. IRWIN and L. J. COLE, 1941 Genic effects on serum proteins. Proc. Nat. Acad. Sci. **27**: 565–570.

1943 Genic control of species-specific antigens of serum. J. Immunol. **47**: 35–51.

GOLDBERG, H. P., and P. F. DE GARA, 1948 On the individual specificity of human serum. J. Immunol. **60**: 555–556.

IRWIN, M. R., 1939 A genetic analysis of species differences in Columbidae. Genetics **24**: 709–721.

1949 On immunogenetic relationships between the antigenic characters specific to two species of Columbidae. Genetics **34**: 586–606.

IRWIN, M. R., and L. J. COLE, 1940 Further studies of the interrelationships of cellular characters in Columbidae. Genetics **25**: 326–336.

IRWIN, M. R., and R. W. CUMLEY, 1942 Immunogenetic studies of species. Qualitative differences in the serum of backcross progeny following a generic cross in birds. Genetics **27**: 228–237.

1947 A second analysis of antigenic differences between species in Columbidae. Genetics **32**: 178–184.

KRUMWIEDE, C., G. COOPER and D. J. PROVOST, 1925 Agglutinin absorption. J. Immunol. **10**: 55–239.

LANDSTEINER, KARL, 1945 The specificity of serological reactions. 310 pp. 2nd ed. Harvard University Press, Cambridge.

PAINTER, T. S., and L. J. COLE, 1943 The genetic sex of pigeon-Ring dove hybrids as determined by their sex chromosomes. J. Morph. **72**: 411–439.

PAULING, L., H. A. ITANO, S. J. SINGER and I. C. WELLS, 1949 Sickle cell anemia, a molecular disease. Science **110**: 543–548.

THE VIABILITY OF HETEROZYGOTES FOR LETHALS[1]

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IN a preliminary report STERN and NOVITSKI (1948) presented data on the viability of individuals of *Drosophila melanogaster* heterozygous for recessive sex-linked lethals. At that time 33 different lethals had been tested. Since then the data have been enlarged and now embrace a total of 77 different X-chromosomal lethals.

THE LETHALS

All lethals arose in males from the Canton-S stock, a strain of flies which had been made isogenic, by the use of marker and crossover suppressor chromosomes, by C. B. BRIDGES, and had gone through renewed crosses leading to isogeneity in 1939. While no obvious heterogeneity has been encountered in the stock, apart from a very low number of visible mutations and the lethal mutations some of which form the material for the viability tests, it is realized that heterogeneity must have been present in the stock during the course of the experiments. As will be described below the lethals were kept in stocks derived from crosses of the Canton-S males to "Muller-5" females. These females were not isogenic. All tests were carried out so that heterogeneity in the genetic background was randomized between lethal-carriers and lethal-free controls.

Of the 77 lethals tested for heterozygous viability effects 37 were "spontaneous" from untreated sperm which had been stored in the spermathecae of inseminated females and had served as controls for sperm subjected to ionizing radiations. The other 40 lethals came from sperm which had been irradiated during storage in spermathecae (CASPARI and STERN 1948; UPHOFF and STERN 1949). The total dosage of irradiation administered continuously either over 24 hours or 21 days in the form of gamma rays from a radium needle, amounted to approximately 52.5 r. (The statement in STERN and NOVITSKI (1948) that some of the lethals came from X-ray experiments was in error.) In the experiments of CASPARI and UPHOFF quoted above, the percentage of "control lethals" averaged approximately three-quarters the percentage of lethals obtained in the irradiated series. Therefore, among the "experimental

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lethals" approximately three-quarters must have had in reality the same "spontaneous" origin as the "control lethals." The latter have been designated as *lc*, those from irradiated sperm as *lr*. A specific number accompanying these symbols serves to identify each lethal.

The lethals studied had been defined as lethals by their original discoverers on the basis of the finding that no adult males containing the mutated chromosome had hatched in the first test generation where they had an opportunity of appearing (for a specific exception see CASPARI and STERN (1948) page 79). In the present experiment, as in the earlier ones, occasionally one or a few males with a "lethal" X-chromosome succeeded in developing to the adult stage and appeared as phenotypically normal individuals. As will be explained in the section on "Methods," the finding of normal males among the offspring of F_2 test mothers would result in misclassification of a lethal-carrying mother as being not a carrier. The overall error resulting from such incidences must have been very low in our experiments. During a period of several years of routine transferring and inspecting of the 77 different lethal-carrying stocks, in only seven were "normal" males found and always in a very low frequency. In some of the stocks these "normal" males may have been rare survivors, in others possibly double-crossovers containing the normal allele of the lethal from the "Muller-5 chromosome." There is evidence for occasional survival of "lethal" males since one of the seven lethals referred to permits development through the pupal stage and half-way emergence from the pupa case. There were six additional lethals in which no normal male had been found during transfers of stocks but in which partly emerged males were observed and, in a test culture, an occasional survivor hatched completely. Finally, two more lethals without survivors in the stock and without partly emerged individuals in pupa cases, produced two test cultures with single normal-appearing males, in addition to numerous "Muller-5" males. Altogether 15 out of 77 lethal strains occasionally showed "normal" males, and in seven of these death of lethal males occurred usually in the pupa case. Most of the normal males in the other eight strains may well have been very rare double crossovers.

It may be safely assumed that the great majority of the chromosomes with spontaneous (control) lethals originally carried a lethal change at only a single locus. Similarly, the chromosomes with the lethals which were induced by low dosage irradiation, probably had a change in a single chromosome region only, although some of these may represent changes affecting several adjacent loci. It must be realized, however, that the lethal-carrying chromosomes were kept in stock for considerable periods before being tested for viability. During these times new lethal mutations might have arisen spontaneously; so that lethal-carrying chromosomes may possibly contain more than one lethal.

The frequency of the occurrence of chromosomes with more than one lethal can be estimated. If the stocks had been carried on by means of a single lethal-carrying female per generation, then, assuming a spontaneous mutation rate

of sex-linked lethals of .2 percent and an average interval of 50 generations between occurrence of the original lethal event and the viability test, about eight lethal stocks out of 77 would be expected to have accumulated a second lethal. Since the stocks were carried on by means of a rough average of twenty females per generation more than eight lethal stocks had a chance of accumulating a second lethal in some of their X-chromosomes. Random selection of females for continuation of the stock would have subjected this mixture of chromosomes with only the original and with two lethals to drift, and thus may have led to loss of the double lethals in some of the stocks. In others, drift might have led to fixation, while in still others a mixture may have been retained. Altogether, only few stocks would be expected to carry chromosomes with more lethals than the original one.

Linkage tests to localize the lethals in 11 stocks were carried out after completion of the viability tests. As will be described in detail, in ten of these stocks the tests indicate presence of a single lethal. In the eleventh stock the lethal action was associated with an inversion involving a considerable section of the chromosome.

Two of the 77 lethals were found to produce at times a morphological anomaly in adult heterozygous females. These two lethals, *lc 5* and *lr 70*, one of which came from a control and the other from an irradiation experiment, will be treated separately from the main group of 75 lethals.

METHODS

The cultures were raised in incubators kept at 26 ± 2°C. In some tests, however, there were short periods in which the temperature fluctuated beyond these limits. The usual molasses-cornmeal medium enriched with dry brewers' yeast was used for these cultures, and Tegosept-M was used to keep down mold growth. The flies whose viability was tested were females of the constitution " Muller-5 "/*lethal*. " Muller-5 " is an abbreviation for an X-chromosome, synthesized by H. J. MULLER, of the formula sc^{81} *B In-S* w^a sc^8 (for a detailed description see SPENCER and STERN (1948), page 44). The most important features of the Muller-5 chromosome are its two inversions, one included within the other, which permit very little crossing-over throughout the length of the chromosome, and its markers Bar (*B*) eye-shape and apricot (w^a) eye color which permit phenotypic recognition of the chromosome.

Crosses were made so as to obtain for each lethal, simultaneously, as sibs, Muller-5/*l* and Muller-5/+ flies (the normal chromosome was derived from the same Canton-S stock which had been the source of the lethals). These crosses were as follows (using virgin females unless noted otherwise):

P Muller-5/*l* females (from Muller-5/*l* female × Muller-5 male stock) × + males. Forty or fifty pairs of parent flies were used in each half-pint culture bottle.

F_1 *l*/+ females × Muller-5 males. Forty or fifty pairs of parent flies were used in each half-pint culture bottle. After the parent flies had been in

the culture bottle for 4–6 days they were removed. Then a wad of Kleenex or similar paper moistened with a heavy suspension of live yeast in water was partially submerged in the food.

F_2 single females of genotypes Muller-5/*l* or Muller-5/+ × Muller-5 males. These cultures were in one ounce vessels, " creamers."

Depending on the genotype of the F_2 females the F_3 male progeny should consist either of only Muller-5 flies with Bar and apricot eyes (mother, Muller-5/*l*); or of both Muller-5 flies and normal flies with round and red eyes (mother, Muller-5/+).

The two types of F_2 females are phenotypically indistinguishable. Theoretically, they should occur in equal numbers. However, if the heterozygous lethal genotype caused a degree of survival from egg to adult different from that of the homozygous normal, the inequality in numbers of the two types would serve as a measure of the viability effect of the heterozygous lethal. *As an index of viability, the number of cultures from lethal-carrying F_2 females was expressed as a fraction of the number of cultures from lethal-free F_2 females.* This index is equal to one if the viability of the heterozygous lethal is equal to that of the lethal-free females. The index is smaller, or larger than one for lethals with reduced, or increased viability in heterozygotes. Some biological problems regarding the interpretation of viability indexes will be discussed in the section on " The relativity of viability indexes." Statistically, the index as defined above has the disadvantage that chance deviations give an asymmetrical distribution around a true value. Therefore, for various statistical tests to be presented below, a different index has been used, namely the number of cultures from lethal-carrying F_2 females as a fraction of the total number, *i.e.*, of both lethal-carrying and lethal-free F_2 females.

The number of F_2 females tested separately for the determination of the viability indexes varied from 227 to 903 for the initial test of each lethal, with a mean number of 541.8. Sterility reduced the number of successful initial tests to a range from 193 to 874, with a mean of 498.9.

Apart from some of the very earliest experiments care was taken to insure virginity of the F_2 females. Since the brothers of these females included normal males non-virginity could introduce the following source of error: If the female had the constitution Muller-5/*l* and had been fertilized by a normal male, then non-disjunction (of either the primary or secondary type) of her X-chromosome could result in the appearance of normal male offspring and thus would lead to misclassification of the mother as Muller-5/+. No danger of misclassification would arise if the mother had the genotype Muller-5/+. Thus, non-virginity coupled with non-disjunction would have led to an underestimate of the viability of lethal-carrying females.

The initial scoring of the test cultures was usually done between the eleventh and fifteenth day after mating of the parents. If during the inspection one or more red and round eyed males were seen, the F_2 female was classified as Muller-5/+, and the culture discarded. If no red and round eyed male was seen, the culture was reinspected between the fifteenth and twenty-first day

after initiation. If again no red and round eyed males were observed although the culture contained a specified minimum number of Muller-5 males, the F_2 female was classified as Muller-5/*l*, and the culture discarded.

An explanation is in order for the term " specified minimum number of Muller-5 males." This number was six in the earlier stages of this investigation but nine later on. This change in specification was due to a change in the number of Muller-5 parental males used per F_2 culture, which first was one but later, for reasons of assuring better fertility, was three. The parent flies were not removed from the cultures before the hatching of the offspring and the large scale inspection of cultures did not make feasible an attempt to classify separately the Muller-5 males into parents and offspring. During the period when a minimum of six Muller-5 males (and no normal males) placed a culture into the lethal-carrying class, these six males may have been either all offspring, or may have consisted of five offspring and one parent. Later, when a minimum of nine Muller-5 males (and no normal males) placed a culture into the lethal-carrying class, these nine males may have been either all offspring or may have consisted of six to eight offspring and three to one parent individuals.

Frequently, no red and round eyed males were found in a culture, but also fewer than the specified minimum number of Muller-5 males were observed. Cultures of this type sometimes were those in which only few offspring had been produced; but more frequently they were cultures in which there were many offspring, but the food had become soft, and in consequence the majority of the flies had died and disintegrated beyond sufficient recognition. Usually no further tests were made with these cultures and they were classified as " doubtful." A considerable number of such cultures, however, was kept for an additional period to permit hatching of more flies including those of a second generation. If normal males appeared, assignment of the culture could be made to the non-lethal-bearing class, while if many Muller-5 males and no normal males developed, assignment to the lethal-bearing class was made. In many of these held-over cultures no normal and not enough Muller-5 males hatched to remove them from the " doubtful " class. In a further attempt to determine the constitution of parent females of doubtful cultures, heterozygous Muller-5 F_3 females were used for new tests. These tests then served to assign the correct constitution, Muller-5/*l* or Muller-5/+, to the original F_2 test female. Some of these tests again were undecisive and could not serve to remove the original test culture from the " doubtful " class. The interpretation of the doubtfuls will be dealt with below.

Finally, some test females produced no offspring at all, due to the early death of themselves or their mates, or to sterility. All these females are listed as " steriles."

ANALYSIS OF DATA

In table 1 the combined results of the 75 initial tests, based on a total of 40,633 females have been summarized. Twenty-two of the lethals were retested one, two, or three times. Since the retests were made with lethals

selected for specific properties they have not been included in table 1. A record of all tests is presented in table 2.

Sterile tests

An inspection of tables 1 and 2 will show that the frequency of sterile cultures varied greatly from test to test, and that the average frequency of sterile cultures was higher in tests of lethals from irradiated than from control sperm. These facts are mainly or perhaps exclusively the result of variations in technique. The high frequencies of sterile cultures occurred mostly in the earlier period of the work. From the totals of the earlier initial tests, the percentage of sterile cultures was 14.9. Later, when more parental males per F_2 culture were employed than at the beginning, the average percentage of sterile cultures decreased to 2.4.

The higher frequency of sterile cultures among *lr* lethals than among *lc* lethals is mainly the result of the "accident" that in the earlier period the number of *lr* lethals tested was about double that of the *lc* lethals tested; while

TABLE 1

Combined results of initial tests of 40,633 females, heterozygous for, or free from one or the other of 75 lethals.

No. and source of lethals	Females heterozygous for		Doubtful	Sterile
	Lethal	Non-lethal		
36 from control sperm (*lc*)	9230	9838	184	1013
39 from irradiated sperm (*lr*)	8648	9200	318	2202

in the later period the reverse was true. The mean percentages of sterile cultures in these two periods were 15.7 and 3.5 for *lr* and 13.1 and 1.8 for *lc*.

By their very nature the sterile cultures did not permit assignment of the F_2 females to either the lethal or the normal-containing genotype. As far as the sterility was caused by non-functioning of the males it may be assumed that the group of females remaining without offspring represented a random sample of the population. Some of the sterility encountered must have been due to non-functioning of the females themselves. If these females also were a random sample of lethal-bearing and lethal-free individuals, then the validity of the viability indexes which were calculated without regard to the existence of sterile cultures, is not affected by omission of these cultures. If, however, the sterile cultures contained a disproportionate number of females of one or the other genotype, then the interpretation of the viability indexes would have to be changed. Instead of indicating the relative number of lethal-bearing flies which have reached maturity the indexes would signify the relative number of lethal-bearing flies which have reached maturity and produced offspring.

TABLE 2

Results of viability tests for individual lethals which comprise initial tests, repeat tests, and means. Symbols for females with these X-chromosomes: l = Muller-5/lethal; non-lethal = Muller-5/non-lethal; df = either l or non-lethal and fertile (see text); st = either l or non-lethal and sterile. n = l + df + non-lethal. $\Delta = \lfloor (100)(l + df)/n \rfloor - (100)(.5n)/n$. $\sigma = 50/\sqrt{n}$. *Percent df as calculated for column 4 was based on fertile tests only. The values in column (2) have been adjusted to the nearest first decimal place. Among others, those with an asterisk (*) have been adjusted upward. Values accurate to two decimal places were used in figures 2, 3, and table 5.*

Lethal strain	Viability index $\frac{l + df}{non\text{-}lethal}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lc 21	1.312	3.4	351	0.32	6	622
" "	1.045	1.3	1850	0.41	39	3650
Mean	1.080	2.5	2201	0.40	45	4272
lc 3	1.258	2.8	320	2.65	25	603
lr 12	1.212	2.3	324	0.34	7	595
" "	1.027	0.3	301	0.17	5	596
" "	1.141	1.6	314	0.17	10	591
Mean	1.124	2.5*	939	0.22	22	1782
lr 99	1.189	1.8	233	1.36	50	440
" "	1.065	0.8	285	1.41	40	568
" "	0.947	0.5	192	1.23	76	405
Mean	1.066	1.2	710	1.34	166	1413
lr 98	1.135	1.2	184	4.74	104	380
lr 50	1.119	1.4	311	0	12	589
" "	0.890	1.4	282	0	2	599
" "	0.866	1.7	272	0	15	586
Mean	0.952	1.0	865	0	29	1774
lr 48	1.109	0.8	136	2.57	213	272
" "	0.970	0.3	216	1.97	44	457
Mean	1.019	0.3	352	2.19	257	729
lc 23	1.097	1.1	301	0.52	7	581
" "	1.114	1.4	334	1.23	49	649
" "	1.120	1.7	463	0.45	19	884
Mean	1.112	2.4	1098	0.71	75	2114
lr 83	1.092	0.9	235	0.66	20	456
lc 10	1.090	1.0	304	0	4	583
" "	1.081	0.9	303	0.51	11	589
" "	1.143	1.6	312	0	15	585
" "	0.921	1.3	474	0.10	13	991
Mean	1.034	0.9	1393	0.15	43	2748

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{1 + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lr 10	1.085	1.0*	280	2.48	23	565
lc 13	1.077	0.9	301	1.18	23	594
lc 17	1.067	0.8	320	0	10	620
lr 65	1.059	0.7	303	0	12	589
lr 13	1.053	0.5	211	2.10	30	429
lr 44	1.052	0.5	218	0.70	55	431
lc 19	1.052	0.5	193	2.53	53	396
lr 89	1.052	0.4	154	2.83	68	318
lc 66	1.043	0.4	181	2.93	79	376
" "	0.901	1.0*	155	2.33	133	344
Mean	0.973	0.4	336	2.64	212	720
lr 1	1.040	0.5	328	1.80	31	667
lr 80	1.024	0.2	203	3.04	37	427
lc 24	1.021	0.3	286	0.35	18	570
lr 23	1.018	0.2	228	0.22	14	454
lr 97	1.014	0.1	211	0.94	54	427
lr 45	1.007	0.1	130	1.86	217	269
lr 96	1.004	0.0	214	4.67	14	471
lc 27	1.003	0.0	304	0.65	8	615
lc 25	1.000	0	278	1.57	14	574
lr 95	0.995	0.0	209	1.17	57	429
lc 8	0.990	0.1	296	0.17	4	597
lr 3	0.969	0.4	332	1.02	7	689
lr 20	0.964	0.4	177	2.87	81	383
lc 11	0.964	0.4	293	0.17	2	599
lr 6	0.962	0.5*	273	1.23	29	571
lc 86	0.959	0.4	195	4.14	40	435
lr 34	0.955	0.5*	200	2.33	39	430
lr 91	0.955	0.4	162	1.74	132	344
" "	1.035	0.4	233	0.86	20	466
" "	0.993	0.1	142	2.34	180	299
Mean	0.998	0.0	537	1.53	332	1109

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{1+df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lr 77	0.953	0.5	214	2.18	19	459
" "	0.702	3.4	155	0.78	97	383
" "	1.140	1.3	196	1.84	111	381
Mean	0.917	1.5	565	1.64	227	1223
lr 53	0.953	0.6	276	1.04	10	578
lr 9	0.949	0.6	260	2.99	29	569
lc 99	0.947	0.6	187	5.94	50	438
lc 98	0.944	0.6	216	0.67	33	451
lc 20	0.944	0.7	301	0.16	6	622
lr 68	0.935	0.7	170	4.63	95	389
" "	1.131	0.8	91	2.23	37	179
" "	0.800	2.2	176	0.99	82	405
Mean	0.908	1.5	437	2.67	214	973
lr 47	0.932	0.8	270	0.35	24	564
lr 2	0.930	0.9	317	0.45	29	664
lc 26	0.929	0.9	297	0.48	6	623
lc 2	0.928	0.8	220	0	10	457
lr 4	0.925	1.0*	303	1.09	46	645
lr 54	0.923	0.7	140	1.33	73	300
lc 9	0.920	1.0	285	0.17	4	597
lc 22	0.925	1.2	413	0.80	29	874
lr 94	0.916	0.9	181	1.29	95	389
" "	0.976	0.2	196	0.99	82	405
" "	0.722	3.3	173	1.41	63	427
Mean	0.861	2.6	550	1.23	240	1221
lc 97	0.911	1.0*	204	0.23	56	430
lc 45	0.911	0.6	90	1.04	34	193
" "	1.033	0.3	179	1.91	124	366
Mean	0.989	0.1	269	1.61	158	559
lc 1	0.910	1.1	283	0	7	594
" "	0.986	0.2	291	0	14	586
Mean	0.947	0.9	574	0	21	1180
lc 6	0.910	0.9	191	0.25	9	403
lr 57	0.908	1.0*	180	1.78	91	393

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{1 + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lr 73	0.907	1.0	186	4.20	57	429
lc 28	0.898	1.3	287	0.49	16	613
lc 95	0.881	1.4	233	0.60	84	504
" "	1.012	0.1	165	1.48	152	338
Mean	0.931	1.0	398	0.95	236	842
lc 14	0.879	1.6	265	1.70	23	588
lc 57	0.871	1.4	198	0.92	54	434
lc 7	0.865	1.8	273	0.50	4	595
lc 36	0.864	1.8	272	0.34	8	591
lc 12	0.861	1.8	261	0.87	13	575
lr 93	0.854	1.5*	158	1.69	120	356
" "	0.920	0.9	197	2.31	70	432
Mean	0.890	1.6	355	2.03	190	788
lr 81	0.851	1.6	174	0.78	75	385
" "	0.890	1.3	201	3.87	50	465
" "	0.922	0.8	194	1.67	36	419
Mean	0.888	2.1	569	2.21	161	1269
lc 62	0.840	1.9	220	1.01	94	493
" "	0.936	0.7	184	1.28	99	391
Mean	0.881	1.9	404	1.13	193	884
lc 96	0.838	1.8	173	3.19	96	408
lr 92	0.837	1.8	165	6.00	69	417
" "	0.917	0.9	217	0.87	41	462
Mean	0.878	1.9	382	3.30	110	879
lr 78	0.833	1.9	187	1.86	39	429
" "	0.844	1.7	187	1.88	53	426
" "	1.052	0.6	260	0.97	44	517
Mean	0.914	1.7	634	1.53	136	1372
lc 64	0.823	2.0*	179	1.70	74	412
" "	1.031	0.3	197	0.76	73	394
Mean	0.919	1.2	376	1.24	147	806
lc 4	0.778	3.0	259	0	10	592
lr 30	0.602	6.0*	211	0.87	25	575
" "	0.666	4.8	230	0.17	22	578
Mean	0.633	7.6	441	0.52	47	1153

TABLE 2 (*continued*)

Lethal strain	Viability index $\frac{1 + df}{\text{non-lethal}}$ (1)	$\frac{\Delta}{\sigma}$ (2)	l (3)	% df (4)	st (5)	n (6)
lc 5	0.665	4.7	211	1.98	45	556
" "	0.613	5.9	224	0.99	92	605
Mean	0.638	7.5	435	1.46	137	1161
lr 70	0.452	8.2	145	0.21	14	469
" "	0.490	8.3	195	0	9	593
Mean	0.473	11.7	340	0.09	23	1062

This interpretation of the viability indexes was found to be the more accurate one for at least two of the lethals, one of which belongs to the lethals with morphological effects in heterozygotes. During the localization tests and viability tests, it was found that *lc 3* and *lc 5* showed higher percentages of sterile females than did the other lethals which were tested at the same time. A direct test for *lc 3* showed that while 1000 Muller-5/*lc 3* females gave 10.7 percent steriles; 1000 Muller-5/+ females gave .6 percent steriles. At the same time other tests were made in order to find out whether the sterility and lethal factors were localized in the same region of the chromosome. For this purpose Muller-5/*lc 3* females with an X-chromosome derived from two types of stocks were used. In one type of stock the left end containing the lethal came from *lc 3*, and the right end from Canton-S. The junction point was between the lethal locus (about 1.6) and *cv*. One thousand Muller-5/*lc 3* females from ten strains with such an X-chromosome gave 4.1 percent steriles; while 1000 Muller-5/+ females from six strains with the complementary chromosome containing no lethal, a left end from Canton-S, and a right end from *lc 3*, gave .9 percent steriles. From these results the lethal and sterility factors for *lc 3* seem to be closely associated if not identical. In the case of *lc 5* the high percentage of steriles found was shown to be mainly due to the death of about 25 percent of the females 0–2 days after emergence. This lethal is described further in the section, " Lethals with morphological effects in heterozygotes."

In addition to this evidence that lethals could increase the percentage of sterile Muller-5/*l* females, it was also shown that the percentage of steriles for some lethals could be as low as the control rate of about .6 percent, for ten lethals gave less than one percent steriles in at least one test. And since the best comparison of percentage steriles in controls and lethals must be made from tests run simultaneously, many other lethals with percentages higher than one percent sterile may not have exceeded their unknown control rate.

Whatever the distribution of the steriles in regard to the two genotypes, the index of lethal-bearing fertile to lethal-free fertile females gives a measure of the reproductive fitness of the lethal heterozygotes (assuming, until further study, that the fecundity of the two genotypes is alike).

Doubtful tests

The " doubtful " tests pose a more serious problem than the sterile tests. As described under " Methods " this group of tests is made up of fertile cultures in which no normal males were found but in which the number of Muller-5 males was very small. Such a small yield of males leaves considerable uncertainty as to whether the absence of normal males is due to the lethal-carrying nature of the non-Muller-5 chromosome in the parent female, or whether the female was Muller-5/+ and, by chance, produced only Muller-5 sons. Arbitrarily, cultures with less than six (or, less than nine) Muller-5 males and no normal males were defined as doubtful. This definition introduces an error and opens a question. The error consists in the fact that there is still an appreciable chance for Muller-5/+ females to produce six or more Muller-5 and no normal sons and thus be misclassified as lethal-bearing. If the viability of a Muller-5 male zygote were equal to that of a normal male zygote the chance of a Muller-5/+ female producing six Muller-5 males and no normal males would be $(1/2)^6$ or about 1.6 percent. In reality, counts based on 3655 males from Muller-5/+ females show that under " normal " culture conditions—*not* necessarily typical of doubtful cultures—the viability of Muller-5 males was only 87 percent of that of normals so that the chance of misclassification in the case of six Muller-5 males is reduced to $(.87/1.87)^6$ or about 1.0 percent. With more than six Muller-5 males, and no normal males, the danger of misclassification of Muller-5/+ females becomes exponentially smaller, being $(.87/1.87)^n$ where n = number of Muller-5 males. Since only a small fraction of any test series—estimated as never more than 1.5 percent and usually very much less—fell into the class of six or more, or respectively nine or more Muller-5 males, and no normal males, the error of misclassification of normal-carrying females is negligibly small.

The question which is opened by the definition of cultures with less than six, or less than nine, Muller-5 and no normal males as " doubtful " is the following: What fraction of the doubtful tests should be assigned to the Muller-5/*l* and what fraction to the Muller-5/+ class? This question may be approached from two different angles, the theoretical and the empirical. Theoretically, it can be calculated how many cultures with 0, 1, 2, 3, 4, and 5, etc., Muller-5 males and no normal males would belong to one or the other of the two classes. Obviously, the chance of a culture with no normal males belonging to the Muller-5/*l* and not to the Muller-5/+ type would increase with increasing number of Muller-5 males present. Therefore, the relative numbers of cultures with from 0 to 8 Muller-5 males would be a determining factor in calculating the over-all partition of the doubtful tests into the two categories. More specifically, however, the calculation would not only depend on the number of Muller-5 males present in a culture, but also on other factors. The partition of the doubtful culture group would depend on such factors as (1) the " true " ratio of lethal and non-lethal-bearing cultures in each lethal test, (2) the proportion of doubtful creamers which was due to low egg laying

activity of the F_2 female, (3) the proportion of doubtful creamers which was due to the death and disintegration of most of a large number of adults because of unfavorable conditions before the creamer was to be checked, (4) possible selective factors in adult death, (5) possible differential effect of the presence or absence of the lethal F_3 genotypes on the conditions which give doubtful creamers. Since little is known about these various factors, tests were made in order to determine directly how many cultures classified as doubtful in the main test belonged to each of the two genotypic classes. Five such tests are available (table 3). Test group 1 consisted of 620 doubtful cultures which were simply kept for an additional number of days so as to permit hatching of further flies usually including F_4 individuals. In 220 cultures so few flies were observed that these tests remained in the doubtful class. In the other 400 cultures sufficient flies appeared to assign 365 cultures to the lethal-carrying and 35 to the non-lethal genotypes.

TABLE 3

Results of progeny tests of doubtful cultures.

Test group	Total retests	Original F_2 female		Remained doubtful	%	%
		Lethal-bearing	Non-lethal-bearing		1	1 + df
		1	non-lethal	df	1 + non-lethal	total
1	620	365	35	220	91.3	94.4
2	202	193	3	6	98.5	98.5
3	116	109	4	3	96.5	96.6
4	67	61	6	0	91.0	91.0
5	201	191	9	1	95.5	95.5
Totals and means	1206	919	57	230	94.2	95.3

In test groups 2 to 5, heterozygous Muller-5 F_2, F_3, F_4, or F_n females from doubtful cultures were mated to Muller-5 males in order to test for production of normal males. Corresponding to the genotype of the non-Muller-5 F_2 females the non-Muller-5 females in any one of the doubtful cultures all had to be either Muller-5/*l* or Muller-5/+, and the absence or presence of normal sons in the new cultures could thus serve for final identification of the until then doubtful tests. As seen from table 3, some of the progeny tests yielded again so few Muller-5, and no normal males that the cultures still remained doubtful. The great majority of the tests were successful. Excluding those cultures which had remained doubtful, from 91.0 to 98.5 percent were assignable to the lethal-bearing genotype, with an average of 94.2 percent. If one assumes that the cultures which had remained doubtful were all lethal-bearing then the total percentages of the lethal-bearing genotypes varied equally between 91.0 and 98.5. It is probably true, that 91.0, the lowest percent, is an underestimate and 98.5, the highest percent, an overestimate of the true frequency of lethal-bearing cultures among the initially doubtful cultures of which the great

majority were never tested further. It is obviously an even greater overestimate to assume that 100 percent of the initially doubtful cultures are lethal-bearing.

In the following sections use will be made of the two extreme estimates, 91 and 100 percent. These two estimates will be employed in connection with different analyses and in each case that estimate will be chosen which leads to the more conservative interpretation of the relevant data.

Tests with less than one percent doubtful cultures

Among the 75 initial test groups of the lethals there were 35 in which the fraction of doubtful cultures amounted to less than one percent of each test group. The mean percentage for 24 control lethals, *lc,* was .36; for 11 irradi-

TABLE 4

Mean viability indexes based on the sums of: A. 35 initial tests with less than one percent doubtful cultures; B. 40 initial tests with more than one percent doubtful cultures; C. all 75 initial tests. P, based on a chi-square test, signifies the probability that the deviation of the index from one is a chance deviation. 100 percent of doubtfuls assumed to be lethal-carrying.

Test group	Type of lethal	Viability index lethal + doubtful / non-lethal	n	P
A	24 strains of lc	.9509	13,742	.003
	11 strains of lr	.9748	5,729	.35
	Total	.9579	19,471	.003
B	12 strains of lc	.9721	5,510	.29
	28 strains of lr	.9744	12,437	.16
	Total	.9737	17,947	.08
C	36 strains of lc	.9569	19,252	.002
	39 strains of lr	.9746	18,166	.09
	Total	.9654	37,418	< .001

Homogeneity tests for sums of tests A versus tests B: lc, P = .49; lr, P = .99; totals, P = .44. Homogeneity tests for sums of lc versus lr: A, P = .44; B, P = .92; C, P = .39.

ated lethals, *lr,* was .45; and for the total was .39. This set of 35 test groups might be regarded as particularly reliable. Assuming that all (100 percent) doubtful cultures were lethal-bearing, the viability indexes (lethal + doubtful/non-lethal) of the 35 lethals range from 1.312 to .602. The mean viability index calculated from the sum of all lethal-bearing (plus doubtful) and all lethal-free cultures is .9579 (table 4, A). Based on 19,471 cases this mean index represents a highly significant decrease from the viability of the non-lethal-bearing females.

A separation of the 35 lethals into those derived from control males and those from irradiated males yields .9509 mean viability for the sums of tests of the 24 control lethals, and .9748 percent for those of the 11 irradiated lethals. The deviation of the first value from one is highly significant, but that

of the second value is not. There is, however, no significant difference between the mean values for the two subgroups (P = .44).

It may be concluded that the presence of a lethal, in heterozygous condition, reduces the viability of a female by several percent. This conclusion is based on using tests with a minimum number of doubtfuls and assuming that all doubtfuls were lethal-carriers.

The viability indexes as given in table 4 are calculated on the assumption that the lethals are a homogeneous group with equal effect on viability of heterozygotes. This assumption permitted use of the simple statistical tests which showed the significance of a decrease in viability for heterozygotes. It will be shown below that the lethals are not a homogeneous group in respect to viability, thus confirming the finding of a heterozygous effect. The mean decrease of viability of the different lethals will be shown to be similar to that represented by the indexes of table 4.

Complete tests

In table 4, C, a summary is given of all 75 initial tests regardless of the frequency of doubtful cultures. These 75 tests include the 35 tests treated in the preceding section. The mean percentages of doubtful cultures were as follows: 36 *lc*, .96 percent; 39 *lr*, 1.75 percent; total, 1.34 percent. Assuming again that all doubtful cultures are lethal-bearers, it is seen in table 4, C, that the mean viability indexes for the total and the *lc* and *lr* groups are very similar to those for the partial data presented in table 4, A. The decreases in viability from one for the total and the 36 *lc* tests are again highly significant, that for the 39 *lr* tests only at the nine percent level. There is no significant difference between the means of the *lc* and *lr* groups (P = .39).

The similarity of the mean indexes for the total of 75 lethals and those for the 35 selected lethals is, of course, partly due to the fact that the latter are included in the data for the whole set of lethals. However, the mean indexes for the 35 lethals with less than one percent doubtful tests are also very similar to those of the 40 lethals with more than one percent doubtful tests (table 4, B). Since these mean indexes are so similar to each other it is concluded that the assignment of all doubtful individuals to the lethal-carrier class gives a close approximation to reality. Therefore further analysis will be based on all 75 lethals.

The viability index for the total, .9654, signifies an average decrease of viability for a heterozygous lethal-carrier of approximately 3.5 percent.

The distribution of viability indexes

The decrease of viability of lethal-carriers is not caused by one or a few specific lethals with strong effects in heterozygotes, but is a characteristic of the group of lethals as a whole. This will be demonstrated by a consideration of the distribution of the 75 viability indexes from initial tests.

Figure 1 gives the distribution in terms of the indexes themselves. They vary from .602 to 1.312. If the presence of a lethal in heterozygous constitu-

tion would not affect the viability of the carriers there should be an equal number of observed indexes which are larger and smaller than the standard value of one. If only one or a few lethals were responsible for the mean decrease of viability the expected equality of indexes above and below one should not be greatly disturbed except for a surplus of one or a few extreme low deviants. However, inspection of figure 1 shows a general shift of the population of lethal indexes toward less than one with a peak in the .925–.974 class.

Both control and irradiated lethals share in this shift and show very similar distributions; although the indexes for the irradiated lethals show a slight shift to higher values than those for the control lethals. A statistical test for determination of the significance of this apparent difference was made by com-

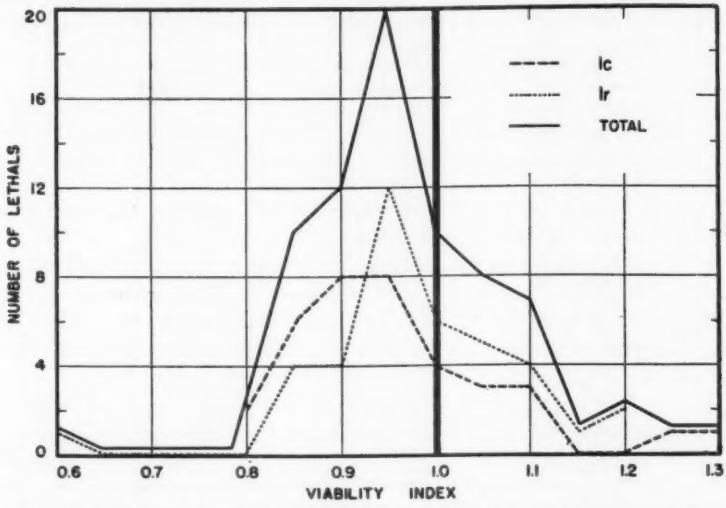


FIGURE 1.—Distribution of viability indexes from 75 initial tests. 100 percent of doubtfuls assumed to be lethal-carrying.

bining the indexes for the *lc* and *lr* lethals each in four classes (1) those below .875, (2) .875 through .974, (3) .975 through 1.074, and (4) 1.075 and above. The chi-square for homogeneity is 1.79 which with three degrees of freedom corresponds to a P value of about .6. Thus, the two distributions do not seem to differ significantly from each other, and the *lc* and *lr* lethals will not be treated separately henceforth. The shift of the distribution of the 75 lethals toward a mean of less than one is statistically significant. This may be shown in various ways.

A simple test consists in comparing the observed number of lethal indexes above and below one with the expected number if one were the mean of the indexes. Obviously, chance expectation would assign equal numbers of lethals to the two classes, namely 37.5. Actually 47.5 lethals gave indexes below and

27.5 above one. Chi-square is 5.33, corresponding to P = .02, strongly indicating significance of the uneven distribution around one.

More detailed tests were based on the distribution of the lethals in terms of standard deviations from an expectation of one (table 5). The data show again a striking shift of the distribution of indexes toward values of less than one. Chi-square tests were performed with several sets of grouped data. In one of these, ten classes were distinguished with intervals of $.5\sigma$ except for bracketing together all classes with deviations of $+2\sigma$ and greater and -2σ and greater: $\chi^2 = 25.05$, d.f. = 9, P = .005. Another test was based on five classes, comprising deviations of -1.5σ and greater, -1.49σ through $-.5\sigma$, $-.49\sigma$ through $+.49\sigma$, $.5\sigma$ through 1.49σ, and 1.5σ and greater: $\chi^2 = 17.57$, d.f. = 4, P = .003. In the two preceding tests the smallest expected classes were 3.31 and 5.02, respectively. In order to avoid an overestimation of significance, a third test was made in which all classes with deviations of + 1.0 and greater, and – 1.0 and greater were bracketed together, thus making all class frequencies greater than 11. This grouping still yielded: $\chi^2 = 14.28$, d.f. = 5, P = about .015. Thus, the tests leave no doubt that the reduced viability of lethal-carriers is an attribute of the group of lethals as a whole and not of a few exceptional ones, particularly since the two lowest indexes deviating respectively 3.04σ and 5.96σ from expectation had not been given their due weight but been merged with less extreme indexes in each one of the different tests.

TABLE 5

Distribution of viability indexes from 75 initial tests in terms of standard deviations from an expected value of one. 100 percent of doubtfuls are assumed to be lethal-carrying. n = *lethal + doubtful + non-lethal;* $\sigma = 50/\sqrt{n}$; $\Delta = [(100)(\text{lethal} + \text{doubtful})/n] - (100)(.5n)/n$. *The expected numbers of lethals are based on the normal frequency distribution.*

$\frac{\Delta}{\sigma}$	0.00–0.49	0.50–0.99	1.00–1.49	1.50–1.99	2.00–2.49	2.50–2.99	3.00–3.49	3.50–3.99	4.00–
Observed positive	9.5	10	4	1	1	1	1	0	0
Observed negative	9.5	18	8	10	0	0	1	0	1
Expected	14.36	11.23	6.89	3.31	1.24	0.37	0.08	0.02	0

On the other hand the tests do not exclude the possibility that not all lethals decrease the viability of carriers. Actually with respect to their viability indexes the 75 lethals do not behave as a homogeneous group. A homogeneity test, with 74 degrees of freedom, yields a chi-square value of 130.31 which corresponds to a P value of less than .001.

In the preceding sections mean viability indexes were calculated from the sums of all lethal and non-lethal-carrying females of the different lethals, but in view of the heterogeneity of the different lethals in their viability effects the best estimates for the effect on viability would be provided by the means of the indexes. Calculating the means of the indexes " lethal-carriers/total " and

expressing the results in terms of "lethal-carriers/lethal-free" these indexes were .9526 for 36 *lc*'s, .9750 for 39 *lr*'s and for the total of 75 lethals, .9642.

These indexes are based on the limiting assumption of 100 percent doubtful cultures being lethal-carrying. Using the most likely assumption of 94 percent doubtful cultures being lethal-carrying (table 3) the average index for all 75 lethals becomes .9607. It signifies an average decrease of viability by 3.9 percent per lethal.

Viability indexes over one

Among the 75 viability indexes obtained in the initial tests there were 27.5 which were higher than one. These high values could be caused by any one

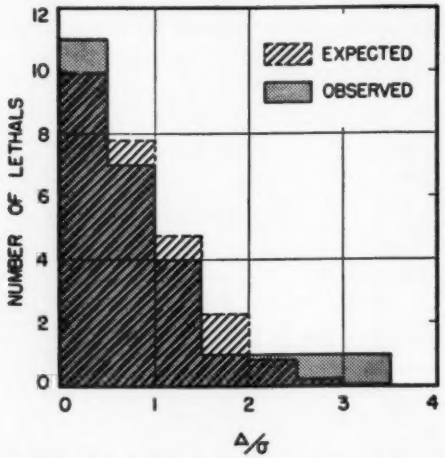


FIGURE 2.—Distribution of (lethal + doubtful)/n indexes from 26 initial tests with (lethal + doubtful)/non-lethal indexes greater than one. Distribution in terms of standard deviations from an expected value of one. 91 percent of doubtfuls are assumed to be lethal-carrying. n = lethal + doubtful + non-lethal; $\sigma = 50/\sqrt{n}$; $\Delta = [(100)\ (\text{lethal} + \text{doubtful})/n] - (100)\ (.5n)/n$. The expected numbers of lethals for each class are based on the normal frequency distribution.

of the following phenomena: (1) chance deviations from true values lower than one, (2) chance deviations from true values of one, (3) occurrence of true values higher than one.

It can be expected *a priori* that some of the higher-than-one values are due to phenomenon (1) but the question arises whether this phenomenon can account for all these values. In order to be on the safe side the indexes over one were recalculated using the assumption that only 91 percent of the doubtful tests actually belonged to the lethal classes. This recalculation reduces the indexes slightly and removes 1.5 out of the 27.5 indexes from the group over one, leaving 26 such values. The distribution of these values in terms of their standard deviations from one is given in figure 2 together with a distribution

based on an expected value of one. Inspection of the figure shows a general shift of the observed distribution, as compared to the expected, toward the left, that is toward a mean of less than one, except for the three indexes which in terms of the standard deviations deviate the most from one. Their observed frequencies, one for each, are higher than the expected frequencies .86, .25, and .06, respectively.

The difference between the three extreme viability indexes and an index of one are, respectively, 3.35, 2.69, and 2.32 times their standard errors. The probability of obtaining, in a sample of 26 indexes three or more which deviate as much or more than 2.32 σ is .015. It must be stated that this determination of probability is biased in two opposite ways. On the one side, by making, *a posteriori*, 2.32 σ the limiting deviation, P tends to be too small; on the other side, by not giving full weight to the two deviations 2.69 σ and 3.35 σ, P tends to be too large. It is not clear how far these two opposing tendencies balance each other, but it seems probable that the 26 indexes over one include indexes whose true value is greater than one. Further evidence for this contention will be presented in the following sections.

The distribution of the 26 indexes is interpreted to mean (a) that the shift toward less than the expected values signifies the presence of indexes with true values lower than one; (b) that the observation of extreme, high values in greater numbers than expected signifies the occurrence of true values higher than one. These two interpretations assume the existence of phenomena (1) and (3) listed in the first paragraph of this section. Since the indexes of the 75 lethals are distributed over a wide range there is no reason to exclude the additional existence of phenomenon (2), namely of indexes with a true value of one. In other words the class of 26 values over one is regarded as a composite of true values below, at, and above one. The proportions of these three groups are unknown, particularly of the most interesting one, that with better than normal viability.

Retests

General. The viability indexes of 22 of the 75 different lethals were determined in more than one test. As seen in table 2, single retests were carried out for 11, two retests each for ten others, and three retests for the remaining lethal.

The lethals chosen for retests cover the whole range of viability indexes (table 2). However, they do not form a random sample since a deliberate selection was made giving some preference to lethals with extremely low and high indexes. Homogeneity tests (table 6), for each group, of initial tests and

TABLE 6

Probabilities that initial and repeat viability tests for individual lethals were homogeneous. There were two to four tests for each of 22 lethals.

P	>.5	.49–.10	.09–.05	.049–.010	<.01
No. of lethals	5	13	2	0	2

single or multiple retest, show that 20 of the 22 lethals gave results compatible with the assumption that the variations of viability indexes within each group were due only to chance ($P \geqq .05$). Two lethals gave homogeneity probabilities of less than one percent: *lc 21,* $P = .0096$; *lr 77,* $P = .006$. The causes for these heterogeneities are unknown. They may involve the presence of a second lethal in some of the tested chromosomes (see page 438). In the case of *lc 21* they may be related to the peculiar emergence pattern of the heterozygous lethal females (see page 441). In addition to the 22 morphologically recessive lethals, retests of the two lethals with dominant morphological effects, which will be discussed below, both gave homogeneity probabilities of $> .5$.

On the whole, the homogeneity of retests of 20 out of 22 lethals shows the reproducibility of the results. These facts deserve special mention since many retests were carried out by different ones of the authors than those who made the initial or a preceding retest. For six of the 11 lethals with two tests each, the second test was carried out by different workers than those who did the first; for four of the ten lethals with three tests each, different workers were in charge of each of the three tests and for four of the ten lethals with three tests each, one of the tests was carried out by a different worker than the one responsible for the other two. The remaining eight retest groups had no alternation of workers. It may be added that often a period of many months or even several years separated a retest from an earlier determination.

Retests of lethals with viability indexes below one. Among the 22 retested morphologically recessive lethals, 14 had decreased viability for both the initial test and the mean of all tests. The result of homogeneity tests within each of 13 out of 14 groups of retests shows the reality of the deleterious effect of these lethals in heterozygotes.

Retests of lethals with viability indexes over one. Eight of the 22 retested morphologically recessive lethals initially had increased viability. When the average indexes were calculated for each of the eight groups of repeated tests, regressions became apparent which removed two lethals from those with an index over one. Five of the six remaining lethals, *lr 48, lc 10, lr 99, lc 23,* and *lr 12* had average indexes of 1.011, 1.034, 1.061, 1.109, and 1.123 respectively (91 percent doubtfuls = lethals). The differences between these values and one are respectively .15, .86, 1.11, 2.38, and 2.45 times the standard error calculated for an expectation of one. Clearly the estimates for the first three of the five lethals do not differ significantly from one. The last two (*lc 23* and *lr 12*) are greater than one at the .0174 and .0142 levels of significance, respectively.

The mean viability index for the sixth lethal, *lc 21,* was 1.078 (91 percent doubtfuls = lethals) which is 2.46 times the standard error ($P = .007$). As stated earlier, the initial test and the retest of *lc 21* differed significantly from each other. It seems safer, therefore, not to make use of the initial test of 622 females whose homogeneity had not been studied, but to use the second test only. It comprised 3650 females which consisted of 12 groups derived from 12 single lethal-carrying females, which came themselves from a single mother. The results of the 12 sub-tests were highly compatible with each other (χ^2 for

homogeneity = 7.64; d.f. = 11; P = .74). The index for the second test was 1.043, which is 1.28 times the standard error (P = .10).

The values of significance for *lc 23* and *lr 12* cannot be taken as proof of the existence of viability indexes over one. They are the extreme deviants in a population of 24 indexes over one which are composed of the 18 indexes from initial tests which were not repeated and the six average indexes greater than one in repeated tests. The deviations of the 24 indexes from one, in terms of their standard deviations are distributed as shown in figure 3. This distribution differs from the one shown earlier for the 26 initial indexes over one in that it accentuates the deviations of the observed distribution from that ex-

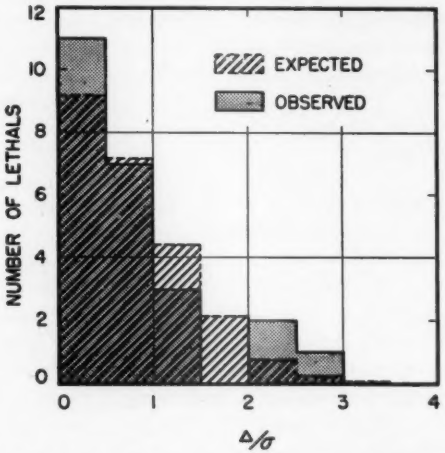


FIGURE 3.—Distribution of (lethal + doubtful)/n viability indexes from 24 complete tests with (lethal + doubtful)/non-lethal indexes greater than one. Distribution in terms of standard deviations from an expected value of one. 91 percent of doubtfuls are assumed to be lethal-carrying. n = lethal + doubtful + non-lethal; $\sigma = 50/\sqrt{n}$; $\Delta = [(100)\ (\text{lethal} + \text{doubtful})/n] - (100)\ (.5n)/n$. The expected numbers of lethals for each class are based on the normal frequency distribution.

pected: the shift to the left, of the bulk of the observed indexes, has increased slightly while the group of three indexes deviating from one by more than 2σ has remained. The changes resulted in a discontinuity so that the class deviating from one by $1.5–1.99\sigma$ is not represented at all. This accentuation of heterogeneity, after the retests, in the group of indexes over one is to be expected if this group consists of true indexes (a) below or (b) equal to one on the one side, and (c) above one on the other. Retests within sub-groups (a) and (b) would shift the average indexes to the left, including removal of some of them from the group over one, while retests within sub-group (c) should retain their status as deviating more than by chance from one.

The deviations of the three extreme indexes *lc 23, lr 12,* and *lc 3,* in terms of σ, are 2.38, 2.45, and 2.69. The probabilities of deviating as much or more

from one are .0174, .0142, and .0072, respectively, among indexes greater than one. Testing for the probability of obtaining, among 24 indexes over one, three or more which deviate by as much as or more than an amount corresponding to a probability of .02, one obtains a P value of .012. In this determination of P the *a posteriori* limit of .02 has been set considerably above the least empirical deviate chosen with its probability of .0174, and no sufficient weight is given to the greater deviates. It is, therefore, believed that the treatment establishes the existence of viability indexes over one.

The data are weighted against finding true indexes over one. The retests of lethals with initial indexes over one included only one lethal with an initial deviation of less than .67 σ as against seven lethals with a deviation of more than .67 σ. Had more of the slightly deviating lethals been retested the number of final indexes over one would probably have decreased to considerably less than 24 without affecting essentially the frequency of extreme indexes. In addition all indexes over one are based on the underestimate of only 91 percent of doubtful tests belonging to the lethal class. A higher estimate would have raised the indexes.

Before discussing the implications of the establishment of increased viability indexes for lethal-heterozygotes, a possible operational, as opposed to an intrinsic, cause for their occurrence will be dealt with.

The problem of two linked lethals

The rather long period of time over which the lethal-carrying stocks were kept, before and during the viability tests, provided an opportunity for new lethals to arise spontaneously in the lethal-bearing X-chromosomes. The consequences of the presence of more than one lethal in an X-chromosome on the viability indexes require evaluation. The discussion will be restricted to consideration of two lethals in a chromosome.

Assume the presence of two lethals, l^1 and l^2, in one chromosome and the presence of the normal alleles in the other X-chromosome of an F_1 female from the cross Muller-5/l females times normal males. Let this $l^1 l^2$/++ female be mated to a Muller-5 male. From this mating the following types of female offspring will be obtained:

(a) non-crossovers $l^1 l^2$/Muller-5, ++/Muller-5
(b) crossovers l^1+/Muller-5, +l^2/Muller-5

Let p be the recombination value for l^1 and l^2, v the mean of the true viability indexes for each lethal when present separately, and cv the heterozygote viability of the two lethals if present together. Then the frequencies of the two non-crossover types will be $cv(1-p)/2$ and $(1-p)/2$ respectively, and the frequency of the sum of the crossover types vp. Since the viability tests as carried out in the present investigation can distinguish between two classes only, namely, (1) the three genotypes with one or two lethals, and (2) the lethal-free genotype, the following "apparent" viability index, v′, is calculated from the tests:

$$v' = \frac{cv(1-p) + 2vp}{1-p} = v\left(c + \frac{2p}{1-p}\right) \qquad (1)$$

Thus, the apparent viability v′ is a function of three parameters, namely, (1) the mean true viability of the two lethals, v, (2) the coefficient, c, which designates the relative viability of the double heterozygote, and (3) p, the recombination value of the two lethals. Perhaps the most interesting fact which the formula expresses is that the apparent viability v′ is larger, by the addition of $2vp/(1-p)$, than the true viability cv of the double heterozygote. Since $2p/(1-p)$ increases with increasing values of p, the value of the apparent viability may rise over one independently of the true value.

These relations may be explored further by finding the "critical" recombination value p′ at which the apparent viability v′ becomes one. It follows from formula (1) that for any value of $p < p'$, v′ remains below one, but for any value of $p > p'$, v′ rises above one. Substituting $v' = 1$ in (1):

$$p' = \frac{1-cv}{1-cv+2v} \qquad (2)$$

This relation is plotted, in figure 4, for a family of curves selected for various values of the true viability v. To illustrate, the line for $v = .961$ shows that at $c = 1.04$ two lethals must be absolutely linked ($p' = 0$) in order to give $v' = 1$, while at $c = 0$ (= lethality of the double heterozygote), p′ is about 34 percent. For $c = .9$, p′ becomes 6.23 percent and for $c = .5$, p′ is 21.04 percent. Or, to choose a much lower true viability $v = .5$, it is seen that for $c = 1$, p′ is 33 percent; for $c = .5$, p′ is 42.9 percent; and for $c = 0$, p′ is 50 percent.

The bearing of these deductions on the results of the viability tests reported in this paper is manifold and depends on the empirical facts regarding v and c. It is reasonable to assume that the majority of the lethal stocks studied contained only a single lethal, and that the true heterozygote viability of most lethals taken singly lies between 1 and .7 with an average of .961 as determined from the tests. It may be assumed further that interaction effects between two lethals in heterozygous state are relatively small and, if present, usually toward lowered viability of the double heterozygote. This places c at values of the order of the true viability of that lethal of a pair which has the lower viability of the two. If c is taken as .7 it appears that for the range of v from 1 to .7 an apparent viability over 1 will be found whenever the lethals are located far enough away from each other to give recombination percentages over 13 to over 26.7.

Having dealt with the critical apparent viability $v' = 1$, we may now consider an extreme case of low apparent viability, $v' = .6$. For this value equation (1) yields:

$$p'' = \frac{.6-cv}{.6-cv+2v} \qquad (3)$$

For $p > p''$, v' would be larger than .6. Choosing a value of $v = .9$ it can be seen that p'' approaches 0 when $c = .66$ and rises to 7.7 percent when $c = .5$. Choosing $v = .7$, p'' approaches 0 when $c = .85$ and rises to 15.2 percent when $c = .5$.

The various relations outlined in this theoretical treatment suggest the need for localization data on the lethals. Theoretically any one of the lethal stocks

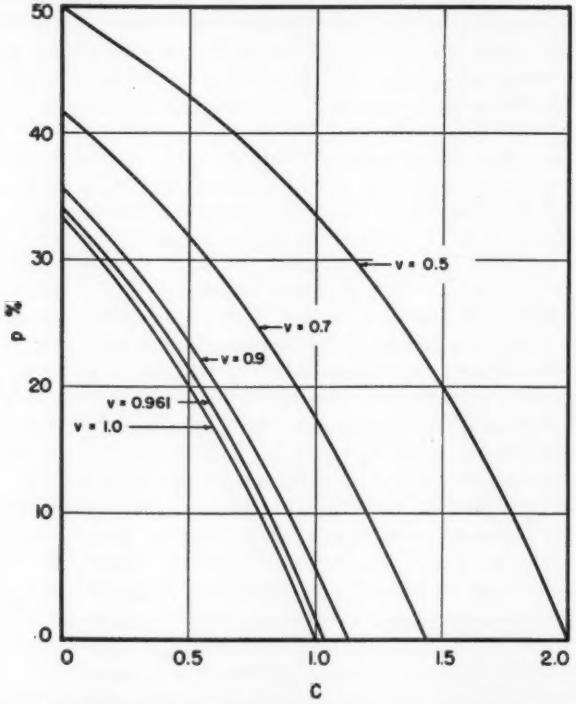


FIGURE 4.—Relation between the apparent viability index, v', and the recombination frequency, p, for two linked lethals with five different values of v, the mean of the true viability indexes for each lethal when present separately: c = coefficient of joint heterozygote viability of two lethals. cv = true viability index for lethals when present together. For given values of v and c the associated value of p represents that recombination frequency at which $v' = 1$. For larger p, $v' > 1$; for smaller p, $v' < 1$.

might contain two lethals in its tested X-chromosome and *a priori* such double lethals might be expected to be distributed at random over the 75 stocks. It has been shown, however, that such a random distribution might result in a nonrandom distribution of the observed viability indexes: On the one hand, low coefficients c for double heterozygotes may yield low apparent viability indexes if the two lethals are located close to one another; and on the other hand, high apparent viability indexes over one may result if the two lethals

are located far away from one another. This latter rather unsuspected deduction is of particular importance since it has a bearing on the interpretation of the observed viability indexes over one.

Localization tests

In view of the theoretical considerations regarding the problem of linked lethals 11 lethal chromosomes were selected for localization experiments: namely, (a) five with high viability indexes, (b) three with low viability indexes, and (c) three with intermediate viability indexes. In a first set of tests all lethals were localized with respect to the four genes y (0.0), cv (13.7), v (33.0), and f (56.7) by classifying the male offspring of *y cv v f/l* females. These tests showed that ten of the eleven lethal chromosomes had their lethal effect localized in one, and only one, of the three regions marked by *y cv v f*. One lethal chromosome, *lr 30,* behaved differently as will be shown below.

If the lethal action of a chromosome is restricted to a single crossover region it is, of course, still possible that more than one lethal locus is present within this region. The existence of two (or more) lethals would usually result in an apparent reduction of recombination between the two marker genes (as determined from the male offspring) since all male zygotes with crossovers between the lethals would die. Normal, or increased recombination values between the two marker genes of a lethal region were, therefore, considered as indicators of the presence of a single lethal only, or at least lethals so closely linked that little crossing over occurred between them. When decreased recombination values between the marker genes were observed the same genes were sometimes followed in test crosses in which the recombination values could be determined from the female offspring. Since the existence of multiple and separable lethals in a region would not reduce the recombination values as determined from the female offspring these tests could show whether the reduction in recombination frequency appeared only from the male offspring or from both sexes. In the former case presence of multiple lethals, in the latter a condition reducing the actual frequency of crossing over in the lethal region was indicated. It should be noted that observed deviations from standard recombination values frequently were not statistically significant although perhaps suggestive.

By means of additional crosses some of the lethal chromosomes were subjected to localization experiments within more narrow limits than those defined by *y cv v f*. The following brief reports on the localization tests list (1) designation of lethal, (2) mean viability index (100 percent doubtful = lethal), (3) region of lethal action, (4) increase or decrease in recombination of marker genes in region of lethal action as determined from female or male offspring (*e.g.,* decrease, ♀ ♀ = decrease determined from female offspring).

(a) Lethal chromosomes with indexes over one.

lc 3. 1.258. *y-spl,* within two units to right of *y*. Decrease, ♀ ♀ (two experiments), ♂ ♂ (three experiments).

lr 12. 1.124. *ct-v,* at about 25. Increase, ♂ ♂.

lc 23. 1.112. *y-w,* within .5 units to right of *y.* Decrease, ♂ ♂.

lc 21. 1.080. *ct-v,* within two units to right of *ct.* Increase, ♂ ♂ (two experiments); decrease, ♀ ♀.

lc 10. 1.034. *y-w,* within .5 units to right of *y.* Decrease, ♂ ♂.

None of the tests suggest the presence of more than a single lethal locus. While the tests cannot exclude the presence of more than one separable lethal locus they demonstrate that such loci would have to be closely linked to each other. Such close linkage is not compatible with an interpretation of the observed viability indexes over one as being spurious in the sense of being caused by the presence of separate lethals with frequent crossing over between them. It is concluded that the observed viability indexes are true indexes of intrinsic properties of heterozygous lethals.

(b) Lethal chromosomes with low viability indexes.

lr 30. .633. *y-cv-v,* crossing over *y-cv* reduced to one percent, *cv-v,* reduced to 11 percent. Salivary gland chromosomes show an inversion from 4 B/C to 8 C with possibly a short deficiency within the inversion.

lc 4. .778. *v-f,* within one unit to left of *f.* Decrease, ♀ ♀, ♂ ♂.

lc 12. .861. *v-f,* at about 37. Decrease, ♀ ♀, ♂ ♂.

The lethality of the three chromosomes with low viability indexes is either due to a single locus or to closely or absolutely linked lethal loci.

(c) Lethal chromosomes with intermediate viability indexes.

lc 22. .925. *cv-v,* at about 27. Increase, ♂ ♂.

lc 1. .947. *cv-v,* within one unit to right of *cv.* Increase, ♂ ♂.

lr 50. .952. *v-f,* at about 45. Decrease, ♂ ♂.

The data on *lc 22* and *lc 1* are compatible with the existence of single lethals. The third lethal stock, *lr 50,* may possibly have contained a double lethal.

The possibility cannot be excluded that at the time of the viability tests some lethal stocks contained a mixture of chromosomes with a single and with more than one lethal, but that at the somewhat later date of the localization tests only chromosomes with a single lethal were encountered. It is, however, unlikely that this should have happened in every one of the eleven cases in which localization tests were made. On the other hand, the low homogeneity probabilities encountered in repeated tests for viability indexes of two stocks (*lc 21* and *lc 77*) may have been in part caused by the presence of more than one lethal in some, but not all chromosomes. The initial test of *lc 21* with its high index 1.31 is particularly suggestive in this respect.

Lethals with morphological effects in heterozygotes

As indicated earlier, two of the total of 77 lethals studied were found to cause morphological abnormalities in heterozygous females. Both greatly reduce the viability of heterozygotes.

lr 70. The morphological penetrance and expressivity of this lethal are variable. It affects the wing, by shortening, blistering, and causing abnormalities

of the veins. Localization experiments place *lr 70* between *v* and *f*, and more specifically in the region which includes *g* (44.4) and *sd* (51.5). The phenotype and localization suggest allelism with *Bag* (*Bg*, 51.6) as described in BRIDGES and BREHME (1944). A considerable reduction of recombination was observed both in the region occupied by the lethal and adjacent to it. Inspection of salivary gland nuclei showed normal chromosomes.

The frequency of aberrant flies depends on temperature. At 26°C Muller-5/*lr 70* females gave an average of 41 percent morphological penetrance; at 17°C this penetrance was only four percent. Moreover, the expression of *lr 70* at the higher temperature was more extreme than at the lower temperature. Concomitantly with the morphological penetrance the viability index for *lr 70* varied with temperature. The initial test, at 26°C, gave an index of .452, the lowest index in our series. A retest at 26°C furnished the value of .490. A test at 17°C gave the very significantly higher index of .869. For each index all doubtfuls were considered lethal. Thus, the penetrance for lethal action of the heterozygous lethal was greatly decreased by the lower temperature although much less so than the penetrance for its morphological expression.

lc 5. This lethal was Notch, both in phenotype and localization. Heterozygotes for *lc 5* in one X-chromosome, and *w spl* in the other exhibit the effect of *spl* but not of *w*. Muller-5/*lc 5* females all show the slight delta-like widening of venation typical for Notch. At about 26°C only 46 percent of the heterozygotes showed other effects on the wings, usually slight notching or blistering.

In the initial test the viability index was .665. A retest gave .613.

In *lc 5* the number of sterile test cultures was unusually high, and obviously due to the early death of some of the test females which were found lying on the surface of the food only a few days after they had been collected. In order to analyze the cause of these deaths, females of the constitution Muller-5/*lc 5* or Muller-5/+ which had been stored for a variable number of days before being placed into the test cultures were separated into groups based on the length of the storage period. Then the frequency of sterile cultures was determined for each group. It was found that sterility of 403 females stored for less than two days amounted to 25 percent; while sterility of 895 females stored for two or more days declined to four percent, that is, the 895 females were those which had lived two or more days in storage before they were used in matings. It may be assumed that most of the 25 percent sterile females belonged to that half of the tested flies which were lethal-carriers. The sterility of lethal-carriers in this experiment would thus amount to 50 percent. In another test, involving 217 Muller-5/*lc 5* females from stock, 26 percent had died within the first two days. It thus appears that the low viability of *lc 5* carriers is perhaps wholly due to sterility and early death of the imagos.

Direct viability tests using visible marker genes

Among the lethals whose loci were determined there were two, *lc 23* and *lc 10*, which gave considerably less than one percent recombination with *y*. Using a *y*-carrying X-chromosome which except for a terminal section to the left of the *w* locus was derived from Canton-S, females of the constitution

y +/+ lc 23 and *y +/+ lc 10* were obtained and mated to *y* males. The F_1 females consisted of the two classes *y +/+ l* and *y/y* (plus rare crossovers). The ratio between these two types furnishes a viability index for the heterozygous lethal genotype (a) provided an adjustment is made for the simultaneous difference in heterozygosity *versus* homozygosity for *y*, and (b) provided that no interaction of viability between the *y* and lethal loci occurs. The crosses were done in mass cultures, as described in " Methods " for the F_1 generation of crosses (page 415) except that for this experiment several precautions were taken in order to reduce the number of variables between bottles. The food for all bottles came from the same batch, and 60.0 grams were used for each bottle. In addition, one-half sheet of Kleenex was partially submerged in the food, and one drop of a suspension of live yeast in water was put on the food. The parents for each test were collected from bottles which had been started at the same time. Females for all tests had emerged during the same seven-hour period, and were put in the culture bottles with *y* males when they were about 1.5 to 8.5 hours old. All the *y* males were collected from the same set of bottles at the same time and were 0–4 days old when they were put in the culture bottles. Forty males and forty females were used for each bottle. Five bottles were prepared for each lethal and six for the control. Cheesecloth bottle coverings were used so that, because of dryness, few pupae would form on the upper part of the bottle. Four days after parents had been put into bottles, they were removed within the same 45-minute period for all 16 bottles. The number of female parents which had survived four days ranged from 37–40 (average 38) for the control, 38–40 (average 39) for *lc 10*, and 31–39 (average 36) for *lc 23*. The number of male parents was 34–40 for the control, 27–38 for *lc 10*, and 35–40 for *lc 23*. After the parents were removed, yeasted paper was added to all bottles within one 20-minute period. One and one-half sheets of Kleenex were soaked in a yeast-water mixture (50.0 g wet yeast in 300 cc water), then squeezed almost dry, formed into a globular wad, and partially submerged in the central portion of the food. This very favorable food supply permitted development of large numbers of offspring. In order to avoid losses during collection of the offspring, the bottles were not shaken out in the usual way, but the flies were collected by means of an aspirator, from the ninth through the nineteenth day.

In the control experiment, among the offspring of *y/+* females × *y* males the ratio of *y/+* to *y/y* females (n = 6109) was .9751. This was nearly identical with the ratio of + to *y* males, .9756 (n = 6069). The unadjusted ratio of *y +/+ lc 23* to *y/y* females was .9755 (n = 4587) while that of *y +/+ lc 10* to *y/y* females was 1.0110 (n = 5126). Adjusting the indexes for the lethals to that of the control, and using the indexes carried out to six significant figures (lethal index/control index), the adjusted indexes for *lc 23* and *lc 10* become 1.0004 and 1.0368, respectively.

Both indexes are above one, and in this regard agree with the indexes obtained earlier for the two lethals by progeny tests. With *lc 10* there is also close quantitative agreement for the viability indexes obtained by direct classi-

fication (1.0368) and progeny test (1.034). For *lc 23* the agreement is poorer, namely 1.0004 *versus* 1.112. This difference may be partly due to differences between the chromosomes used in the two kinds of tests. To some degree the difference seems also to be based on a peculiarity of the emergence pattern of heterozygotes for *lc 23*. As will be shown in the next section, in both direct and progeny tests the frequencies of *lc 23* heterozygotes (*y*+/+ *lc 23* and Muller-5/*lc 23*) relative to non-lethal carriers were higher during the first two days of adult emergence than during the following days. Since, in the progeny tests, emerging females were usually collected for a shorter period than in the direct tests where all flies were counted, it is likely that the higher viability index obtained in the progeny tests is a reflection of selection for testing of early emerging flies. The high experimental progeny test "viability index" for *lc 23* must therefore be redefined as an index of increased speed of development exhibited by females heterozygous for this lethal.

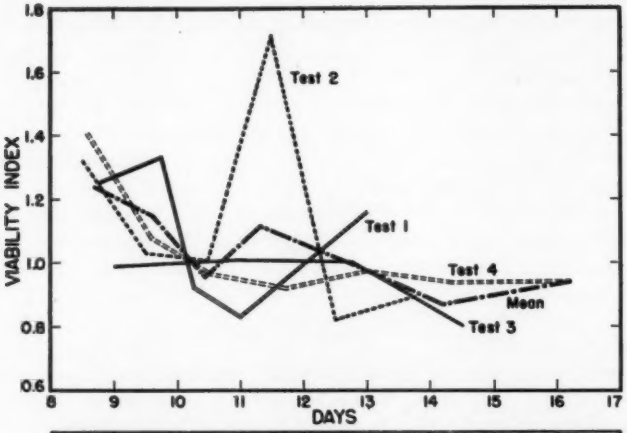
It may be added here that *lc 23* is a lethal which in localization, and phenotypic effect in hemizygous males, seems to correspond to the well known tumor-causing *l(1)7* discovered by BRIDGES. A review of work done on it is given in BRIDGES and BREHME (1944). It thus appears that *lc 23* causes tumors and larval death in hemizygotes, but increased speed of development in heterozygotes.

Relative rates of emergence from the puparium

For some of the experiments all Muller-5/+ and Muller-5/*l* females emerging from puparia in a set of culture bottles were collected. In other experiments only a portion of the total number of females was collected. Whenever only a portion was used, the collections were made at various times during the period when adults were emerging in a set of culture bottles; and in most of these experiments a high proportion of the females were collected within the first four days that adults emerged. Although more than half the total number of flies that could be collected from a bottle emerged during the first four days, it was considered important to investigate whether the proportion of emerging lethal-bearing to lethal-free females, that is, the viability index, remained constant from day to day or whether significant deviations occurred. Such deviations would be expected if, for instance, the time of development from egg to emergence from the puparium differed in lethal-bearing as compared to lethal-free females, or if the change of cultural conditions with time acted selectively on the egg, larval, or pupal survival of the two genotypes.

In numerous cases records of date of emergence were kept for the females tested. Daily viability indexes were calculated for 11 lethals which had had large numbers of flies tested, including the three with best established total indexes over one, *lc 23, lr 12,* and *lc 3,* as well as the two lethals with visible effects. The daily indexes of eight lethals did not show any persistent trend. Lethal *lc 21* showed initially low, followed by high indexes; and *lc 3* exhibited in a more variable way a similar pattern suggesting retarded development. The last lethal, *lc 23,* showed a particularly interesting pattern of changing

indexes, namely, a considerable surplus of lethal-bearing heterozygotes during the first days of emergence, and a slight deficiency in later days (figure 5). This pattern of increased speed of development was found in three separate series of tests including both progeny tests and direct ones. The only striking exception found in these three was a second rise of the index on the twelfth day in one of the test series. A fourth test showed no marked difference from the control until the last collection when there was a sharp decrease in the proportion of lethal-carrying females.



RANK	1	2	3	4	5	6	7	TOTAL
Test 1	124	203	119	108	95	–	–	649
Test 2	188	262	149	122	80	83	–	884
Test 3	967	–	–	725	114	133	–	1939
Test 4	96	893	1031	1128	685	548	206	4587
Mean	1375	1358	1299	2083	974	764	206	8059

FIGURE 5.—Emergence rates: A comparison of females carrying an *lc 23* X-chromosome and females with other types of X-chromosomes. Both types of females which were compared in a test came from the same mass cultures, and had the same parents. Flies were collected at intervals of about 24 hours. Each point marks the viability index for all the flies collected during an interval and the mid-point of the time interval. Rank indicates the sequence of collections, and the rows show the numbers of flies collected for each test. Time was measured in days after the parents were put in the culture bottles. Types of X-chromosomes compared: Tests 1 and 2, *lc 23*/Muller-5 + +/Muller-5; Test 3, *lc 23/y cv v f* + *y/y cv v f*; Test 4, *lc 23/y* + *y/y*.

For a number of lethals, data are available which show emergence rates of Muller-5/*l* relative to Muller-5/Muller-5 females (figure 6). Based on large numbers the rates differ strikingly from one lethal to another. Since the variability within the set of cultures for each lethal was rather small, it appears thus that many lethals have characteristic patterns of emergence which distinguish them from one another.

The findings on specific rates of emergence of heterozygotes for lethals have a bearing on the interpretation of the viability indexes. In experiments in

which all females produced throughout the entire emergence period by F_1 *l*/+ mothers were tested for their genotype the viability indexes are strictly indicators of viability of the two types of F_2 females, regardless of different rates of emergence. When, however, the F_2 females were collected over part of the emergence period only; then the proportions of the two types may be a reflection of the different emergence patterns rather than of true viability. This has been pointed out earlier for *lc 23* whose viability index over one was " redefined as an index of increased speed of development." It is likely that other indexes, over one or below one, also are subject to redefinition in terms of

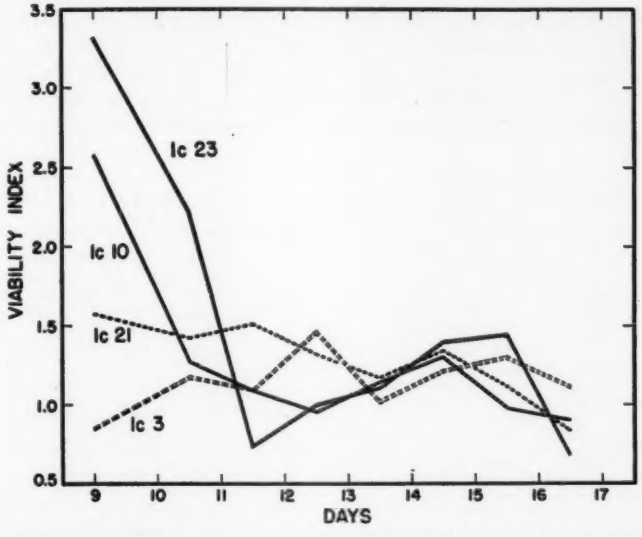


FIGURE 6.—Emergence rates: A comparison between females with both X-chromosomes Muller-5 and females with one Muller-5 and one lethal-bearing X-chromosome. Both types of females which were compared in a test came from the same mass cultures, and had the same parents. Flies were collected at intervals of about 24 hours. Each point marks the viability index for all the flies collected during an interval and the mid-point of the time interval. Time was measured in days after the parents were put in the culture bottles. The numbers of X-chromosomes compared in each test: *lc 23*, 4720; *lc 21*, 4075; *lc 10*, 3310; *lc 3*, 4416. (Viability Index = M-5/*l* ÷ M-5/M-5.)

developmental rates. From the point of survival in natural or artificial populations, relative developmental rates are probably often more significant than more static viability differences.

The relativity of viability indexes

Viability indexes are not absolute measures of the capacities of specific genotypes. Obviously, variations in environment and genetic background would be expected and are known to affect viability. The viability indexes determined in the present study imply an additional variable. Even apart from the influence of relative rates of emergence they do not simply represent the fre-

quency of survival from egg to reproductive period of lethal-carriers in relation to a standard of viability as determined in independent experiments. Rather, the indexes given constitute relative frequencies of lethal-carriers and lethal-free females developing in the same culture bottles. The frequency of lethal-free females in these cultures could be regarded as a general standard, if it could be assumed that the development of the lethal-free females proceeded without being affected by the presence of other genotypes. In reality, this assumption is not justified.

In order to study the problem of possible interaction in viability of different genotypes developing in the same culture, three lethals, *lc 3, lc 10,* and *lc 21,* were selected. All three lethals increased the viability of heterozygotes. F_1 females from the cross, $+/l$ female × Muller-5 male, were mated to Muller-5 males, and single females with three males were placed in creamers. Comparisons were then made between the numbers and types of flies hatched in cultures whose parent females were Muller-5/+ and those which were Muller-5/*l*.

TABLE 7

Mean numbers of offspring in various classes from Muller-5/non-lethal and Muller-5/lethal females × Muller-5 males.

Lethal strain	P ♀ Muller-5/non-lethal							P ♀ Muller-5/lethal				
	No. of cultures	M-5 / + ♀♀	M-5 / M-5 ♀♀	M-5 ♂♂	+ ♂♂	Total	No. of cultures	M-5 / l ♀♀	M-5 / M-5 ♀♀	M-5 ♂♂	Total
lc 3	54	28.4	20.1	23.4	27.8	99.6	21	29.9	22.2	24.7	76.8
lc 10	47	30.7	20.6	21.0	29.1	101.5	37	34.4	26.3	28.6	89.4
lc 21	24	29.0	16.7	19.6	31.0	96.3	20	32.0	23.4	24.9	80.3

The data are summarized in table 7. On the average, the yield of cultures free from lethals was higher than from lethal-carrying cultures. A higher yield was to be expected if it were true that the presence of the lethal male genotypes and of the female lethal-carriers did not affect the development to imagos of the other genotypes. Specifically, under these circumstances the total number of flies hatched in the lethal-carrying cultures should have been approximately 25 percent less than in the lethal-free cultures, the deficiency being accounted for by the death of the male lethal zygotes (the death of a fraction of the female lethal carriers, as well as other genotypes being disregarded). However, contrary to this specific expectation the reduction in total number of flies emerging in lethal-carrying cultures amounted to only 23, 12, and 17 percent for *lc 3, lc 10,* and *lc 21* respectively. All three surviving classes of flies account for this relatively higher yield, but in these experiments the replacement of the lethal genotypes was not complete. Otherwise the yield of the lethal-carrying cultures would have equalled that of the lethal-free ones. Moreover the amount of replacement differed for the three lethals tested. These facts indicate dependence of successful development of non-lethal genotypes on presence or absence of simultaneously present lethal genotypes. Apparently,

a surplus of eggs is laid in the lethal-carrying cultures, and presumably in the lethal-free cultures; but the death of lethal genotypes provides room for the development of greater numbers of the three nonlethal genotypes in the lethal-carrying cultures than was possible in the lethal-free cultures.

Under different circumstances replacement was found to be absent, or on the contrary to reach 100 percent: (1) Absence of replacement was encountered in the direct viability tests described in a preceding section. The average yield of six cultures with $y/+ \times y$ parents was 2029.7; while the average yields of five cultures each of $y+/+$ *lc 23* or $y+/+$ *lc 10* $\times y$ parents were only 1378.0 and 1512.8. Therefore there was 32 percent reduction for *lc 23* and 25 percent reduction for *lc 10* where 25 percent reduction was expected for both if there were no replacement. (2) Very high replacement was observed in other experiments with *lc 3* and *lc 23* (table 8) in which the yield of $+/l$ females was compared to that of control +/+ females, both mated to Muller-5 males. The culture methods used were the same as those described earlier (p. 440) ex-

TABLE 8

Numbers of offspring and sex-ratios from +/+ × Muller-5 and +/lethal × Muller-5. For details see text.

P ♀	Culture no.	Total F_1	♀♀/♂♂
+/+	1	1481	0.87
	2	1541	1.09
	Total	3022	0.97
+/lc 3	1	1281	2.08
	2	1402	1.86
	Total	2683	1.96
+/lc 23	1	1447	1.98
	2	1682	1.99
	Total	3129	1.99

cept for the following changes. For each lethal and for a control two bottles were used. Female parents were all collected within the same 13-hour period. The females and males were put in the culture bottles when the females were 17–30 hours old and the males were 0–2 days old. The parents were left in the culture bottles for five days. The numbers of parents surviving after five days were not recorded. Two pieces of Kleenex were used instead of one and one-half. Collections of flies were made through the seventeenth day.

The three sets of bottles showed the expected proportions of female to male flies, namely, approximately 2 ♀ ♀ : 1 ♂ in the lethal-carrying bottles and 1 ♀ : 1 ♂ in the lethal-free bottles. However, in spite of the death of about one-half of the male zygotes in the lethal-carrying bottles their total yield was only 11 percent less in the *lc 3* bottles than in the lethal-free cultures, and even 3.5 percent more in the *lc 23* bottles.

The existence of the replacement phenomenon accentuates the effect of intrinsic viability differences between the heterozygotes of the different lethals studied and lethal-free females; an intrinsically lower viability of lethal-carriers

than of lethal-free females may result not only in a relative decrease of the former but also in an absolute increase of the latter. Conversely, an intrinsically higher viability of lethal-carriers may not only mean a relative but also an absolute increase of this class. Thus, the different viability indexes obtained in the present study are functions of intrinsic viability differences, of overpopulation, and of competition in the cultures. It is likely that all these factors play a role in natural populations of developing flies. Furthermore, as has been discussed in the preceding section, some of the viability indexes may not be related to viability *per se* but be reflections of specific emergence patterns of lethal heterozygotes.

DISCUSSION

The data presented in this paper have shown that the majority of, if not all, so-called recessive lethals in the X-chromosome of *Drosophila melanogaster* have a dominant effect on viability. Most of the lethals depress viability in heterozygotes as compared to lethal-free females. A few lethals increase viability in heterozygotes. The range of viability indexes of the 75 lethals without morphological effect in heterozygotes extends from about 1.3 to .6. The addition of lethals with variable morphological penetrance in heterozygotes extends the range down to .45.

For the 75 lethals a mean decrease of heterozygote viability of the order of four percent has been calculated. Since this average lumps together lethals with decrease, neutrality (if it exists), and increase of heterozygote viability, it is perhaps more informative to state that most lethals decrease heterozygote viability, and that the observed average decrease caused by these lethals is approximately four percent. A more accurate determination of this value is not available since it is (1) subject to a considerable sampling error, and (2) would depend on an exact, but lacking, knowledge of how many, and which lethals have no effect, or an improved effect on heterozygote viability. The estimate of approximately four percent average decrease is remarkably similar to a value reported by MULLER and CAMPBELL (MULLER 1950a, b, c) for autosomal lethals, induced by ultra-violet light.

The discovery of lethals which increase the viability of heterozygotes over that of lethal-free homozygotes adds further to the now known number of cases with monohybrid heterosis. It is, of course, possible that the increases in viability described in this paper are the results of specific interactions of the heterozygous lethal locus and the complex heterozygosity present in the X-chromosomes at other loci (Muller-5/*l*), as well as with the undefined heterozygosity in the autosomes. Monohybrid heterosis, however, is always developmentally linked up with genic interaction at different loci. While it is possible and even probable that the superiority of the lethal heterozygotes may occur in some genetic backgrounds and not in others, it remains very probable that the difference in the constitution of the lethal locus is the decisive differential.

A possible objection to this statement could be raised. Should the lethal-

carrying X-chromosomes contain genes other than the lethal which would tend to increase the viability of the heterozygote; then the observed viability would not be due to the lethal locus itself. We have no data at present with which to check this point rigorously. It can be stated, however, that such genes would have to be located rather close to the lethal locus in order to produce an observable effect. This is so because there is opportunity for free crossing over between the lethal-carrying and the lethal-free Canton-S chromosomes of the *l*/+ females whose daughters furnish the Muller-5/*l* and Muller-5/+ test females.

The existence of deleterious genes with increased viability in heterozygotes in a population should lead to a higher accumulation of the deleterious allele than would be expected from its ill effects in the homozygote. For the sex-linked lethals described in this report the increased accumulation would be negligible since selection against the lethals in the hemizygote would by far outweigh any positive selection for the heterozygote. However, for autosomal lethals the accumulation effect might be appreciable. This phenomenon has taken on significance in human genetics as a possible explanation for the high allele frequencies for deleterious genes such as those for Cooley's anemia (NEEL 1950).

In recent years the literature on the effect of heterozygotes on viability and on other traits has been reviewed repeatedly. MULLER (1950b, c) has commented on the studies in Drosophila by MASING (1938, 1939), DOBZHANSKY and WRIGHT (1941), BERG (1942), and DUBININ (1946). GUSTAFSSON and his collaborators (GUSTAFSSON 1946, 1947; GUSTAFSSON and NYBOM 1950; NYBOM 1950; GUSTAFSSON, NYBOM, and VON WETTSTEIN 1950) have covered the field even more widely and contributed important new data. It is not necessary, therefore, to discuss the relevant literature once more, except to call attention to TEISSIER's significant studies (1942).

The significance from the standpoint of population genetics of decreased viability effects of heterozygotes of deleterious genes has been treated among others by DOBZHANSKY and WRIGHT (1941), STERN and NOVITSKI (1948), and particularly in respect to human populations, by MULLER (1950a, b, c). MULLER has stressed not only the fact that genetic death due to deleterious genes in heterozygotes will be by far more frequent than in homozygotes, but he has also emphasized that a deleterious gene of slight dominance " would also wreak much the greater part of its damage short of death in heterozygotes " (page 131, 1950b). This may well be true in many cases, and has been discussed above with respect to sterility (*lc 3*), early death of an adult (*lc 5*), rate of development (*lc 3* and *lc 21*), and morphological effects (*lr 70* and *lc 5*). However, impairment of carriers does not necessarily occur with all genes. In the development and functioning of an organism there occur frequently threshold phenomena which result in all-or-none effects. The alternative between death and living is not always one between death and impaired living, but may be one between death and normal life.

SUMMARY

1. Seventy-five sex-linked lethals in *Drosophila melanogaster* were tested for viability effects on females heterozygous for a lethal as compared to females not carrying a lethal (viability of these = 1). None of these lethals showed dominant morphological effects. Thirty-six of the lethals had arisen spontaneously in controls, while 39 lethals occurred in males exposed to about 50 r gamma irradiation.

2. On the average the viability of heterozygotes for lethals was .965 as calculated from an overall total of lethal-carrying and lethal-free females. No significant difference in viability was found between lethals of control and irradiated origin.

3. The various lethals have characteristically different viability indexes ranging in initial tests from .602 to 1.312 with the mode in the .925–.974 interval. The best estimate of the mean viability as calculated from the 75 separate indexes was .961, a decrease of viability of approximately four percent per lethal.

4. Statistical considerations, partly based on retests of specific lethals, established with high probability that several lethals increase the viability of carriers beyond that of noncarriers.

5. Spurious viability indexes over one could be obtained if there were more than one lethal per chromosome. Localization tests eliminated this possibility for the lethals so studied.

6. Two lethals with slight, or variable morphological effects in heterozygotes were also investigated. They showed very low viability indexes. For one lethal the low viability appears to be due to early post-imaginal deaths.

7. The viability indexes obtained depend on competitive survival of lethal-free and lethal-carrying individuals growing up together in the same cultures. In some experiments partial or complete replacement of lethal male hemizygotes by viable genotypes has been demonstrated.

8. Some lethals were shown to change the rate of emergence of heterozygous females, either by slowing down or increasing the speed of development. The latter applies to a lethal which, in hemizygous state, produces tumors and may be identical with BRIDGES' *l(1)7*. The viability indexes obtained in this study are reflections not only of differential survival of lethal-carriers, but also of their different rates of development.

LITERATURE CITED

BERG, R. L., 1942 Dominance of deleterious mutations in the populations of *Drosophila melanogaster*. C. R. (Dokl.) Acad. Sci. **36**: 212–218.

1942 The role of isolation in the evolution of dominance in natural populations of *Drosophila melanogaster*. C. R. (Dokl.) Acad. Sci. **36**: 284–289.

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552**: 257 pp.

CASPARI, E., and C. STERN, 1948 The influence of chronic irradiation with gamma-rays at low dosages on the mutation rate in *Drosophila melanogaster*. Genetics **33**: 75–95.

DOBZHANSKY, TH., and S. WRIGHT, 1941 Genetics of natural populations. V. Relations between mutation rate and accumulation of lethals in populations of *Drosophila pseudoobscura*. Genetics **26**: 23–51.

DUBININ, N. P., 1946 On lethal mutations in natural populations. Genetics **31**: 21–38.

GUSTAFSSON, A., 1946 The effect of heterozygosity on variability and vigour. Hereditas **32**: 263–286.

1947 The advantageous effect of deleterious mutations. Hereditas **33**: 573–575.

GUSTAFSSON, A., and N. NYBOM, 1950 The viability reaction of some induced and spontaneous mutations in barley. Hereditas **36**: 113–133.

GUSTAFSSON, A., N. NYBOM, and U. VON WETTSTEIN, 1950 Chlorophyll factors and heterosis in barley. Hereditas **36**: 383–392.

MASING, R. A., 1938 Increased viability of heterozygotes for a lethal in *Drosophila melanogaster*. C. R. (Dokl.) Acad. Sci. **20**: 173–176.

1939 Different viability among flies of *Drosophila melanogaster* heterozygous for lethals. C. R. (Dokl.) Acad. Sci. **23**: 835–838.

MULLER, H. J., 1950a Radiation damage to the genetic material. Amer. Scientist **38**: 33–59, 126, 399–425.

1950b Some present problems in the genetic effects of radiation. Oak Ridge Symposium on Radiation Genetics, Mar. 1948. J. Cell. Comp. Physiol. **35** (Supp. 1): 9–70.

1950c Our load of mutations. Amer. J. Human Genet. **2**: 111–176.

NEEL, J. V., 1950 The population genetics of two inherited blood dyscrasias in man. C. S. H. Symp. Quant. Biol. **15**: 141–158.

NYBOM, N., 1950 Studies on mutations in barley. I. Superdominant factors for internode length. Hereditas **36**: 321–328.

SPENCER, W. P., and C. STERN, 1948 Experiments to test the validity of the linear r-dose / mutation frequency relation in Drosophila at low dosage. Genetics **33**: 43–74.

STERN, C., and E. NOVITSKI, 1948 The viability of individuals heterozygous for recessive lethals. Science **108**: 538–539.

TEISSIER, G., 1942 Vitalité et fécondité relatives de diverses combinaisons génétiques comportant un gène léthal, chez la Drosophile. C. R. Acad. Sci. Paris **214**: 241–244.

UPHOFF, D. E., and C. STERN, 1949 The genetic effects of low intensity irradiation. Science **109**: 609–610.